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Application News

No. L526

High Performance Liquid Chromatography

Preparative Purification of Ibuprofen and Its Related Substances by Prominence™ UFPLC

Preparative and purification by liquid chromatography is a widely-used technique applied to drug synthesis, finding effective compounds in natural products, and structural analysis of trace unknown compounds in the pharmaceutical, food product, and chemical industries. Prominence™ UFPLC*1 (hereinafter, UFPLC) enables substantial labor-savings in preparative purification by automating not only fractionation of the target compound but also the related processes of concentration, purification, recovery, etc. This article introduces an example of preparative purification of a mixed sample of the pharmaceutical ibuprofen and its analogs by using Shimadzu's UFPLC Advanced System (Fig. 1).

*1 UFPLC: Abbreviation of Ultra Fast Preparative and Purification Liquid Chromatograph

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Fig. 1 Prominence™ UFPLC (Advanced System)

Procedures of 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. Separation of target compounds in complex sample by preparative LC and introduction of them into trapping columns
2. Replacement of solvent in trapping columns with ultrapure water
3. Elution of target compounds from trapping columns by organic solvent

An outline of the respective processes is shown in Fig. 2.

Preparative Purification of Ibuprofen and Its Analogs

Ibuprofen is one type of nonsteroidal anti-inflammatory drugs (NSAIDs) used as a fever-reducing drug and analgesic. The United States Pharmacopeia (USP) provides analytical methods for ibuprofen and its analog 4-isobutylacetophenone, using valerophenone as an internal standard. This article describes preparative purification of compounds these three components using UFPLC (Fig. 3).

Table 1 shows the preparative purification conditions and Fig. 4 shows the preparative LC chromatogram of the mixed solution. The mixed solution was prepared by dissolving the three target compounds with mobile phases to make the content of each component as 5000 mg/L.

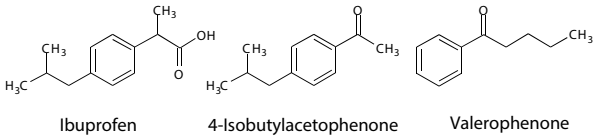

Fig. 3 Structural Formulas of Ibuprofen and Its Analogs

Table 1 Preparative and Purification LC by UFPLC

Preparative LC conditions	
Column	: Shim-pack™ VP-ODS (250 mmL. × 10.0 mmI.D., 5 µm)
Mobile phase	: A: 1 % (wt/v) chloroacetic acid aq. sol. (pH 3.0 adjusted with ammonium hydroxide) B: Acetonitrile A/B = 2/3 (v/v)
Flow rate	: 9.0 mL/min
Column temp.	: Ambient
Injection vol.	: 100 µL
Detection	: UV 230 nm (prep cell)
Rinse conditions	
Column	: Shim-pack™ C2P-H (30 mmL. × 20 mmI.D., 25 µm)
Rinse solvent	: A: 2 % (v/v) acetonitrile aq. sol., B: water
Time program	: A 15 mL/min (0-2 min) → A 8 mL/min (2.01-4 min) → B 8 mL/min (4.01-8 min)
Elution conditions	
Eluent	: acetonitrile
Flow rate	: 4.5 mL/min
Detection	: UV 230 nm (prep cell)

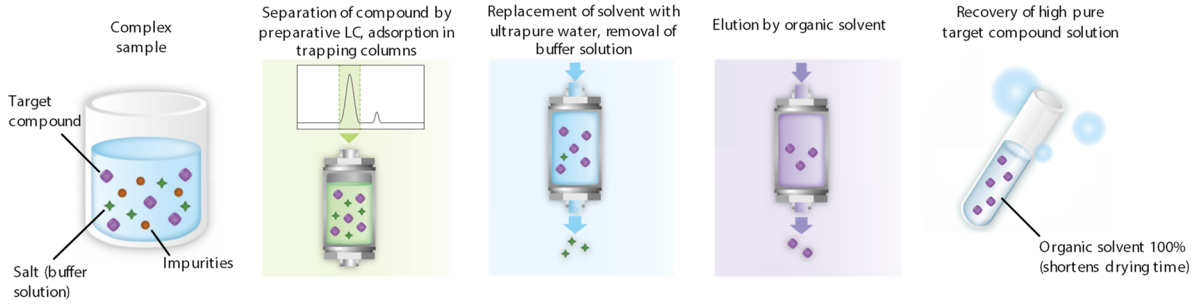

Fig. 2 Flow of Fractionation, Concentration, Purification, and Elution by UFPLC

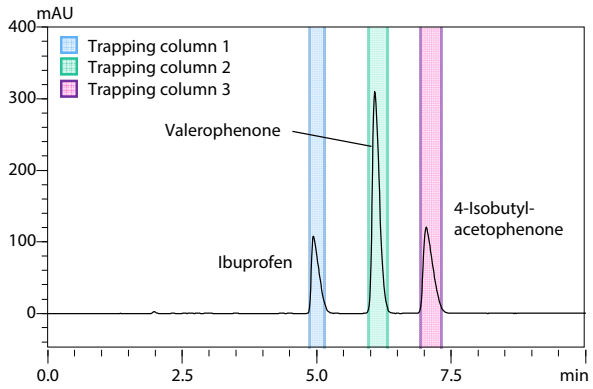

Fig. 4 Preparative LC Chromatogram of Ibuprofen and Its Analogs (UFPLC)

Verification of Purity of Ibuprofen and Its Analogs

The fractions of ibuprofen, valerophenone, and 4-isobutylacetophenone collected by UFPLC were analyzed by Prominence to verify the purity of the compounds. Table 2 shows the analytical conditions and Fig. 5 shows the chromatograms. Table 3 shows the purities of the target compounds contained in each fraction by area normalization at UV230.

Table 2 Analytical Conditions for Purity Verification (Prominence™)

Column	: Shim-pack™ VP-ODS (250 mmL. × 4.6 mmI.D., 5 µm)
Mobile phase	: A: 1 % (wt/v) chloroacetic acid aq. sol. (pH 3.0 adjusted with ammonium hydroxide) B: acetonitrile A/B = 2/3 (v/v)
Flow rate	: 2.0 mL/min
Column temp	: 30 °C
Injection Vol.	: 10 µL
Detection	: UV 230 nm (analytical cell)

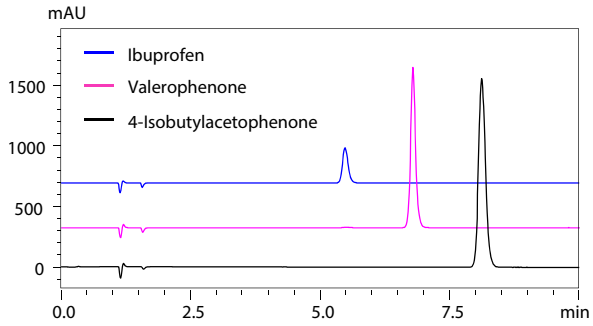

Fig. 5 Chromatograms of Fractions Obtained by UFPLC

Table 3 Purities of Target Compounds Contained in Collected Fractions (Area Percentage, UV 230 nm)

	Area %
Ibuprofen	99.2
Valerophenone	99.6
4-Isobutylacetophenone	99.8

Comparison of Fraction Drying Times by UFPLC and Preparative LC

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 following process. However, an extremely long drying time is required with the conventional reversed-phase preparative LC because the mobile phase contains water. Moreover, in cases where a nonvolatile buffer solution is used in the mobile phase, the salt is sometimes precipitated after drying. In preparative purification using UFPLC, it is possible to remove the nonvolatile salt used in the separation process, because desalting is performed in the trapping columns. The drying time is substantially shortened due to using organic solvent for sample recovery from the trapping columns.

To confirm the reduction of drying time, the fraction of ibuprofen collected by UFPLC was dried with a centrifugal concentrator. The drying time of ibuprofen fraction from preparative LC was also measured and the time lengths were compared. Drying of the ordinary preparative LC fraction required approximately 180 min, however, UFPLC fraction was completed in about 20 min (Fig. 6). Observing respective dried yield, chloroacetic acid and ammonium salt used in the mobile phase were found in the preparative fraction. Consequently it cannot be used for next process, as it is. In case of the UFPLC fraction, crystals of sure ibuprofen were confirmed after drying due to desalination on the trapping column (Fig. 7).

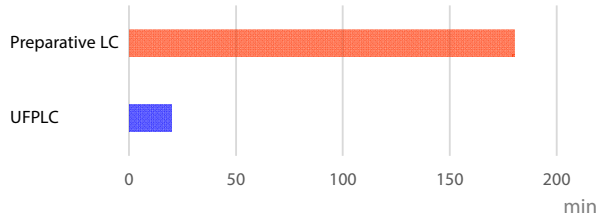

Fig. 6 Comparison of Solvent Drying Time

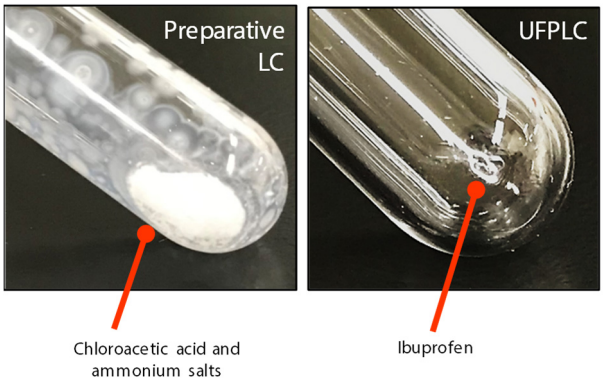

Fig. 7 Conditions of Ibuprofen Fractions after Drying

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