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Automated scale-up system for reversed phase purification - Nexera™ ASAPrep™

Application News

Streamlining of Preparative Purification Work by Nexera ASAPrep

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User Benefits

- Automatic generation of optimal preparative conditions based on a unique scale-up algorithm streamlines preparative purification operations.
- Intuitive UI design and automatic conditioning function allow easy preparative purification, regardless of operator's experience.
- Automatic determination of difficulty level of sample preparation supports assignment of preparative purification work.

■ Introduction

Since impurities as well as main compound are usually generated during compound synthesis, purification is essential to improve the precision of subsequent processes. Preparative purification liquid chromatography (preparative LC) is often used for purification work, but there is a need to improve the efficiency of this process since this work requires expert users and a considerable amount of time.

Nexera ASAPrep (Automated Scale-up from Analytical to Preparative), an automated scale-up HPLC system for reversed-phase purification, is equipped with a function that automatically determines the difficulty level of sample preparation and automatically generates optimal preparative conditions. Furthermore, the intuitive UI design and automatic conditioning function allow anyone to easily perform preparative purification, regardless of operator experience. Through these features, this system can support the assignment of preparative purification tasks based on the difficulty level of preparation, thereby helping to improve the efficiency of preparative purification process.

This article introduces a preparative purification workflow with Nexera ASAPrep, using three mixtures containing the main compounds and related impurities.

■ Preparative purification workflow using HPLC

In preparative purification using HPLC, ideally, the chromatographic separation is evaluated under generic conditions, followed by optimization of the separation and investigation of the maximum loading amounts on analytical scale before confirming reproducibility and adjusting the loading amounts on preparative scale (Fig. 1 green arrow). However, the process of optimizing the separation especially requires know-how and a lot of work hours on preparative HPLC. Therefore, if the above-mentioned workflow is applied to all synthetic compounds that require purification, the overall throughput of the preparative laboratory will be drastically decreased, including also additional work for HPLC expert users.

In preparative purification using HPLC, a shortcut workflow may be taken for the purpose of improving throughput. After analyses under prefixed initial conditions, preparative HPLC conditions are set based on the retention time of the target compound on analytical scale and preparative purification is directly executed (Fig. 1, red arrows). While this flow can be expected to increase throughput because it skips optimizing the separation, it is unavoidable that the risk that the target compound and impurities may be co-eluted during preparative purification. In addition, it is difficult to eliminate human factors from preparative purification process, since the selection of the workflow requires the judgment by preparative HPLC expert to reduce the risk.

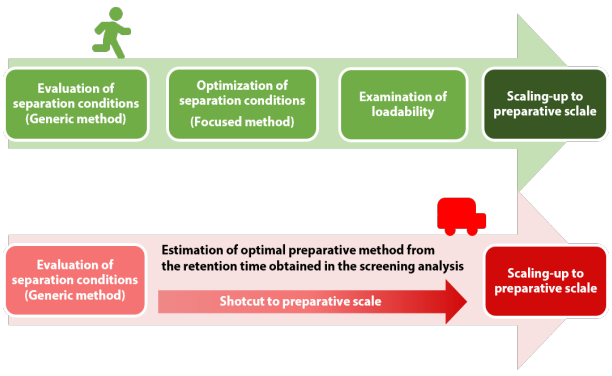

Fig. 1 Ideal preparative purification workflow (green arrow) and shortcut workflow (red arrow)

■ Streamlining of preparative purification workflow using HPLC

Fig. 2 shows a schematic diagram of the automatic preparative scale-up function in Nexera ASAPrep. This system automatically determines the difficulty level of preparative purification based on the results of sample analyses (screening analyses) using two sets of prefixed analytical conditions (analysis time: 2 min) employing acidic and basic mobile phases respectively.

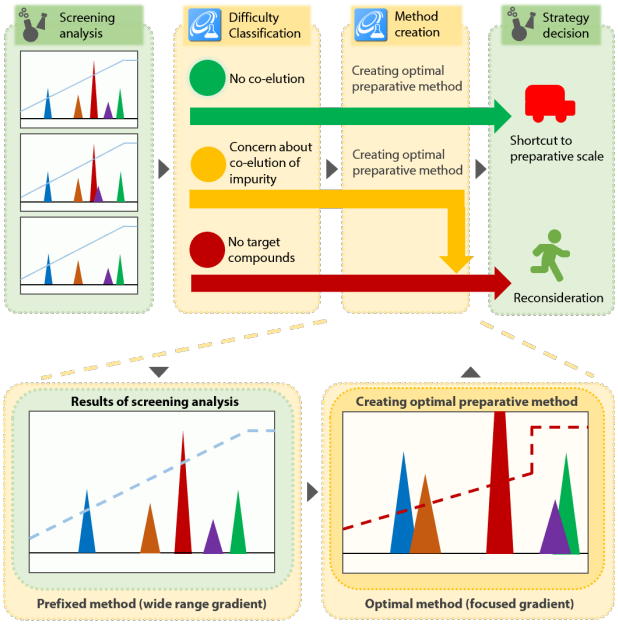

Fig. 2 Autoscaling-up feature of Nexera ASAPrep.

Specifically, there are three levels: when sufficient peak separation of the target compound has been achieved in the screening analyses (green judgment), when there is concern about the presence of impurities during preparative run because sufficient peak separation of the target compound is not achieved in the screening analyses (yellow judgement), and when the target compound is not detected in the screening analyses (red judgement). In the case of green and yellow judgements, the system automatically creates preparative HPLC conditions that optimize the separation near the target peak elution interval based on the results of the screening analyses. Through these functions, the operator only needs to perform screening analyses using the two sets of prefixed conditions provided by the system, then automatic determination of the difficulty level for the preparative separation of the sample and automatic creation of preparative HPLC conditions are performed. For a sample with a green judgment (low difficulty), purification can be performed by shortcutting the process of evaluation of prep conditions on analytical scale without any examination from expert users. For the sample with a red judgement (high difficulty), purification is performed by the preparative HPLC expert from optimizing separation on analytical scale. This allows for easy streamlining of the purification workflow. For a sample with a yellow judgement (difficulty: medium), the automatically generated preparative HPLC conditions can be used as base conditions for further optimization, thus a reduction of workload can be expected.

■ Analytical conditions and target compounds

The screening analytical conditions and target compounds are listed in Table 1 and Table 2, respectively. In this article, screening analyses were performed on a mixed sample containing the main compound and several related substances using the two sets of prefixed analytical conditions, acidic and basic conditions.

Table 1 Screenig analytical conditions

System	: Nexera ASAPrep_analytical system
Column	: Shim-pack Scepter™ C18-120 (50 mm × 3.0 mm I.D., 3 µm*1)
Sample	: Please see table 2.
Sample Concentration	: Main : 10 mg/L
Mobile Phase (acidic condition)	: Pump A : 0.05 % formic acid in water : Pump B : 0.05 % formic acid in acetonitrile
Mobile Phase (basic condition)	: Pump A : 10 mmol/L ammonium hydrogen carbonate in water/acetonitrile = 90/10 : Pump B : 10 mmol/L ammonium hydrogen carbonate in water/acetonitrile = 10/90
Injection volume	: 2 µL
LC Conditions	
Time program	: B.Conc 5 % (0 min)→5-90 % (0-1 min)→ 90 % (1-2 min)
Column Temp.	: 40 ˚C
Flow rate	: 1.5 mL/min
Detection (PDA)	: 190 - 800 nm (SPD-M40, semi-micro cell*2)
MS Conditions	
Ionization	: ESI/APCI (DUIS™), positive and negative
Mode	: SCAN (*m/z* 150-1000)
Nebulizing Gas Flow	: 2.0 L/min (N_2)
Drying Gas Flow	: 5.0 L/min (N_2)
Heating Gas Flow	: 7.0 L/min (N_2)
DL Temp.	: 200 ˚C
Desolvation Temp.	: 450 ˚C
Interface Voltage	: 3.0/-2.0 kV (positive/negative)

*1 P/N : 227-31015-01
*2 P/N : 228-64725-41

Table 2 Main compounds of each mixture

	Mixture A	Mixture B	Mixture C
Main	Vanillin	Indomethacin	Bifonazole

■ Screening analysis

In the screening analyses, after setting the mobile phases and the three mixed samples to HPLC system for screening analyses, the four items of “sample location,” “sample name,” “injection volume,” and “target mass” were set according to the procedure shown in Fig. 3, and the check mark icon was clicked.

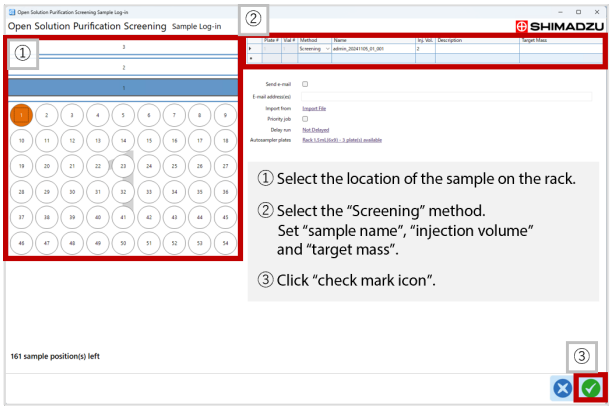

Fig. 3 Setup procedure for screening analysis

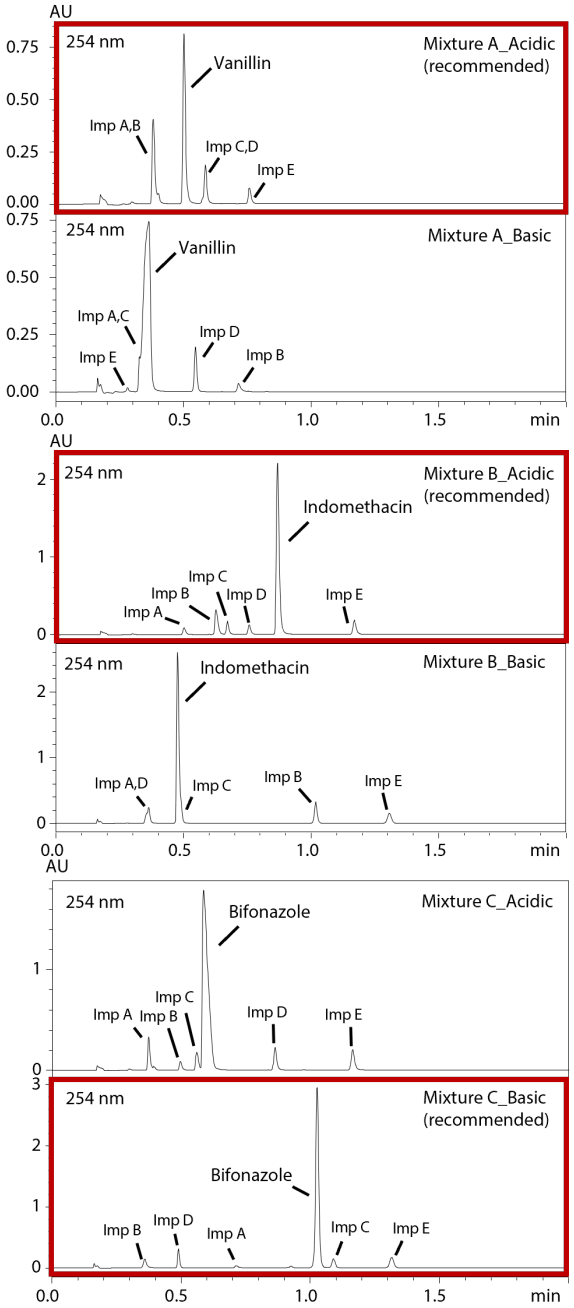

Fig. 4 LC screening chromatograms of each mixed sample
Mixture A (top), Mixture B (middle), Mixture C (bottom)

Sample Name	Descriptio	Mass Found	Masses	Judge	NH4HCO3 RT	NH4HCO3 B.Conc	NH4HCO3 Screening Reason	HCOOH RT	HCOOH B.Conc	HCOOH Screening Reason
C5_Vanillin	sub : C8...	152.1	152.1	HCOOH	0.36	2.4	Below B.Conc threshold	0.5	15.8	Passed
B4_Indomethacin	sub : C4...	357.8	357.8	HCOOH	0.48	12.4	Preferred mobile phase is HCOOH	0.87	47.5	Passed
B16_Bifonazole	sub : C...	310.4	310.4	NH4HC...	1.03	58.2	Passed	0.59	20	Preferred mobile phase is NH4HCO3

Fig. 5 Determination of purification difficulty level based on the screening analyses

By simply setting the above four items and clicking the icon, analysis order is automatically adjusted to minimize the number of conditions witching, and mobile phase replacement and column stabilization are automatically performed when the conditions are switched (automatic conditioning function) as well as consecutive analyses under the two different acidic and basic mobile phase conditions. This makes it easy for the HPLC operators to perform screening analyses.

The HPLC chromatograms obtained from the screening analyses and the results of the determination of preparative difficulty level are shown in Fig. 4 and Fig. 5, respectively. For each sample, the results of the determination of preparative difficulty level and the initial "B. Conc" in the preparative run were obtained by performing the analysis under two different acidic and basic mobile phase conditions in 2 min analysis time. In addition to above-mentioned green, yellow, and red judgments, it is indicated that which mobile phase condition of acidic or basic, should be tried at first, and the reason for the judgment, such as lack of spectral purity of MS peaks. Therefore, even if the result of difficulty determination is only yellow or red, and a further investigation is required, HPLC operator can smoothly proceed with necessary work based on the reasons indicated in the determination results. Determination results and initial "B.Conc" are automatically output as an Excel file.

Table 3 Preparative conditions

System	: Nexera ASAPrep Preparative System
Column	: Shim-pack Scepter C18-120 (150 mm × 20 mm I.D., 5 µm[*3])
Sample	: Please see table 2.
Sample Concentration	: Main : 10 mg/L
Mobile Phase (acidic condition)	: Pump A : 0.1 % formic acid in water : Pump B : 0.1 % formic acid in acetonitrile
Mobile Phase (basic condition)	: Pump A : 10 mmol/L ammonium hydrogen carbonate in water/acetonitrile = 90/10 : Pump B : 10 mmol/L ammonium hydrogen carbonate in water/acetonitrile = 10/90
Injection volume	: 500 µL
LC Conditions	
Time program	: B.Conc x[*4] % (0 min)→x-x+20 % (0-9 min)→ 100 % (9-12 min)
Column Temp.	: ambient
Flow rate	: 20 mL/min
Detection (PDA)	: 190 - 800 nm (SPD-M40, prep cell[*5])
MS Conditions	
MS makeup	: Methanol
Flow rate (makeup)	: 1.5 mL/min
Desolvation Temp.	: 100 ˚C
Interface Voltage	: 3.0/-2.0 kV (positive/negative)

*3 P/N : 227-31102-03
*4 x : Initial B.Conc. of focused gradient
*5 P/N : 228-64727-41

■ Execution of preparative purification

Since the results of the screening analyses indicated that all the mixtures could be applied to preparative purification, and acidic conditions were recommended for the mixture containing vanillin and indomethacin as major compounds, and basic conditions for the mixture containing bifonazole as a major compound, preparative purifications were carried out for all the mixtures. Under respective recommended conditions. Table 3 shows the preparative HPLC conditions. After setting the mobile phase and the three mixed samples in preparative HPLC system, the Excel file containing the results of the determination of preparative difficulty level output at the screening analyses was imported according to the procedure shown in Fig. 6, and the check mark icon was clicked. The mobile phase replacement and column stabilization are automatically performed when mobile phase changeover is required. This allows HPLC operator to perform preparative isolation easily and efficiently.

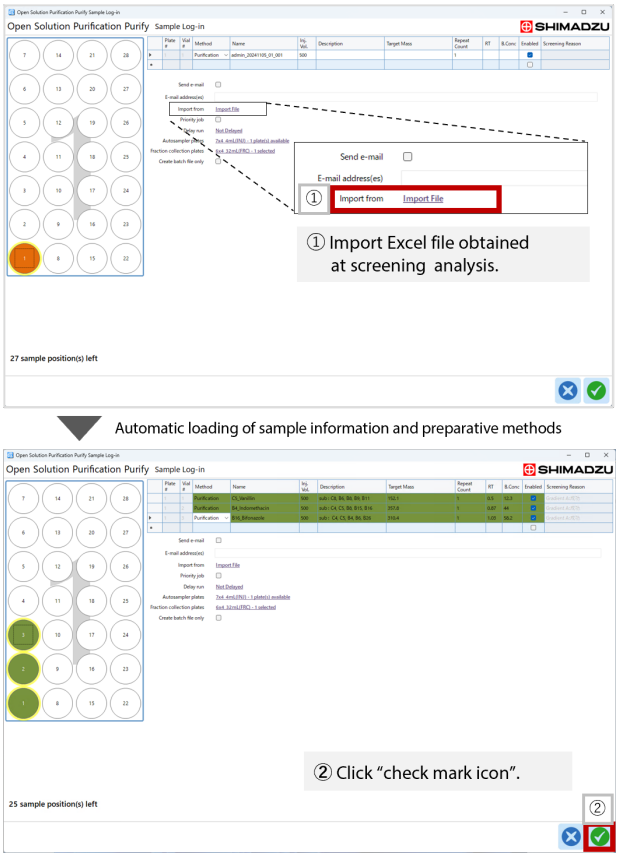

Fig. 6 Setup procedure for purification

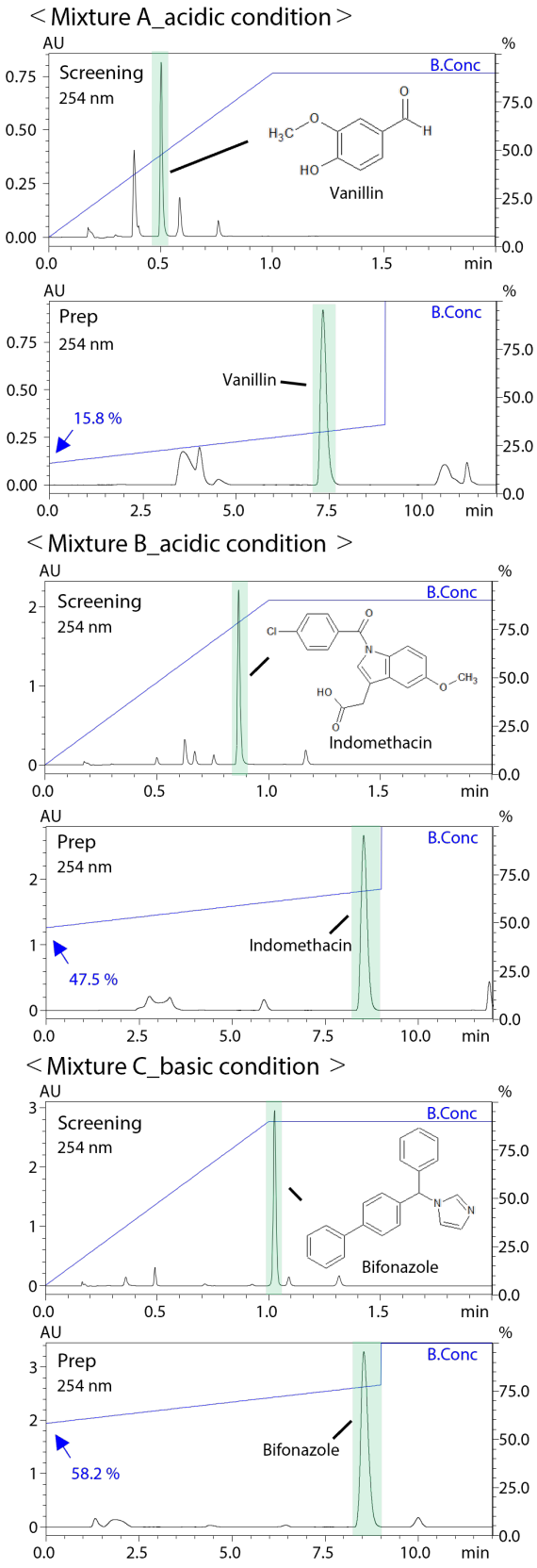

Fig. 7 Comparison of LC screening chromatogram and preparative chromatogram. Mixture A (top), Mixture B (middle), Mixture C (bottom)

Fig. 7 shows the comparison between chromatograms in screening analyses and those in preparative runs of the three samples. The samples with the same concentrations as in the screening analyses were injected to preparative HPLC in 250-fold increased volume, and peaks of the main compounds were separated from the related substances better than those in the screening analyses. Acidic mobile phase condition was recommended for the acidic compounds Vanillin and Indomethacin, and basic mobile phase condition for the basic compound Bifonazole, suggesting that acidic (basic) mobile phase condition would be suitable for the preparative analysis of acidic (basic) compound.

■ Conclusion

A preparative purification workflow using Nexera ASAPrep was introduced. The intuitive UI design, automatic conditioning function, and automatic creation of preparative HPLC conditions provide easy execution of screening analyses and preparative purification, regardless of the experience of HPLC operator. In addition, this system automatically determines the preparative difficulty level of samples based on the screening results, and indicates the risk of co-elution of main compound and related substances on preparative scale as first answer. Through these functions, the system can support the assignment of preparative purification samples according to the preparative difficulty level, thereby helping to improve the efficiency of preparative purification workflow.

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