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Application News

High Performance Liquid Chromatograph Nexera™ FV

On-line Monitoring of Synthetic Reactions Using Nexera FV

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User Benefits

- On-line monitoring of synthetic reactions is enabled using Nexera FV. Simple switching to the Nexera ultra-high performance liquid chromatograph is possible.
- The dedicated software enables automatic sampling according to the specified sampling interval.
- The automatic pretreatment function provides automatic operations from sampling to dilution.

■ Introduction

In the pharmaceutical and fine chemical industries, there is an increasing demand to switch from conventional batch manufacturing, the mainstream method, to integrated continuous manufacturing from drug substance to drug product. Expected benefits of continuous manufacturing include reduced environmental load, increased efficiency (labor and human resources savings), and improved quality and safety. The International Conference on Harmonization (ICH), which discusses international guidelines for pharmaceuticals, has issued ICH Q13, a guideline for the continuous manufacturing of pharmaceuticals in 2022, and this has encouraged active consideration of continuous manufacturing in Japan and abroad. Based on above mentioned reasons, the demand for Process Analytical Technology (PAT) for quality control in each process is increasing. The Nexera FV is an HPLC system equipped with an autosampler and flow through vials for on-line analysis in flow synthesis (Fig. 1). By using flow through vials and an external syringe unit, the system can automatically perform all processes of sampling from a synthesis vessel, HPLC analysis, and report output.

This article introduces an on-line monitoring using Nexera FV, using the esterification reaction of carboxylic acids as a model case.

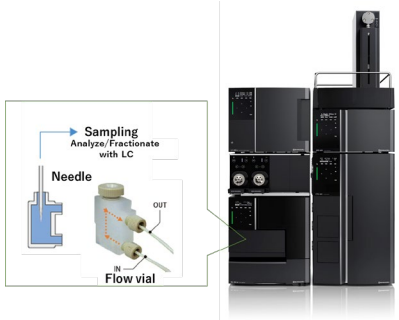

Fig. 1 Nexera™ FV (for batch analysis) and flow through vial

■ Experimental conditions

Phenacyl bromide has a bromoacetyl group and is used as a derivatization reagent for HPLC. It reacts immediately with carboxyl groups to form esters under a basic condition.

In this study, phenylpropionic acid was used as the carboxylic acid for on-line monitoring of the esterification reaction (Fig. 2). A 250 mL acetone solution containing 150 mg of phenylpropionic acid and 398 mg of phenacyl bromide was used as solution A. After heating this solution A to 55˚C, 147 µL of triethylamine as solution B was added dropwise and stirred well.

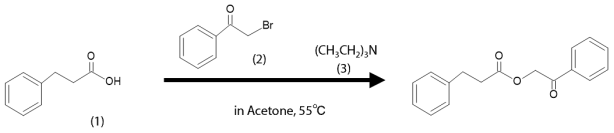

Fig. 2 Esterification reaction of phenylpropionic acid with phenacyl bromide

(1) : 3-Phenylpropionic Acid, (2) : Phenacyl Bromide, (3) : Triethylamine

■ Online monitoring with Nexera FV

Nexera FV is capable of aspirating samples from the synthetic vessel using the external syringe unit (Fig. 3). In this study, the samples were automatically aspirated from the synthetic flask for HPLC analyses according to the created analytical batch. The obtained result before the addition of solution B was employed as the data at “0 min”.

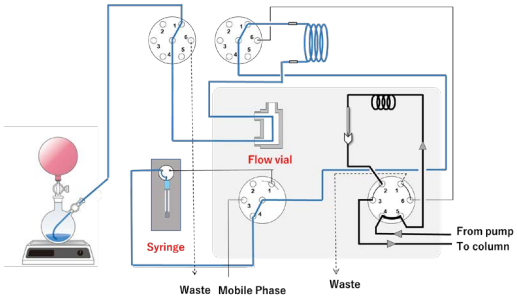

Fig. 3 Aspiration mechanism for sampling from synthetic flask.

■ Easy creation of analytical batch using LabSolutions FV

LabSolutions FV, a dedicated online monitoring software, allows users to easily perform complicated procedures such as creating an analytical batch and entering operational settings during reaction monitoring (Fig. 4). The analytical batch is automatically created by simply entering information such as HPLC conditions and sampling interval, allowing to start online HPLC analysis without any difficulties. Fractionation into vials and dilution operations are also supported. It also supports external signal input and sampling start at a specified time, providing co-operation with other systems.

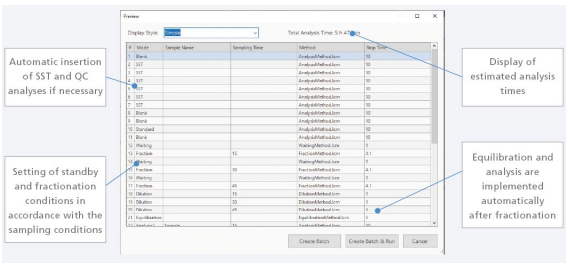

Fig. 4 Parameter setting screen of LabSolutions™ FV

■ Analysis of reaction solution

In this monitoring of the synthesis reaction, HPLC analyses were performed using the direct injection method, in which the reaction solution is aspirated with the external syringe unit and introduced directly into the flow path. Table 1 shows the analytical conditions, and Fig. 5 shows the chromatograms at 15 min. and 300 min. after adding solution B. Peaks of phenylpropionic acid, phenacyl bromide, and the reaction product appeared at 0.49 min, 0.99 min, and 1.4 min, respectively.

Under a basic condition, phenacyl bromide reacted immediately with phenylpropionic acid to form esters. As the decrease of phenylpropionic acid, the yield of the product was increased. After about 50% yield progress in 60 min, the reaction speed slowed down to obtain about 85% yield in 300 min. Phenylpropionic acid had an absorbance of about 1/28th of that of the product in terms of area, but this did not significantly affect the yield.

Table 1 HPLC analytical conditions

System	: Nexera FV
Column	: Shim-pack™ Velox C18*1 (50 mm × 2.1 mm I.D., 1.8 µm)
Temperature	: 40 ˚C
Injection volume	: 1 µL
Mobile phase A	: 0.5 % formic acid in Water
Mobile Phase B	Acetonitrile
Flow rate	: 0.4 ml/min
Time program (%B)	: 30% (0 min)→95% (0.45-1.40 min)→30% (1.41-3.5 min)

*1 P/N： 227-32001-02

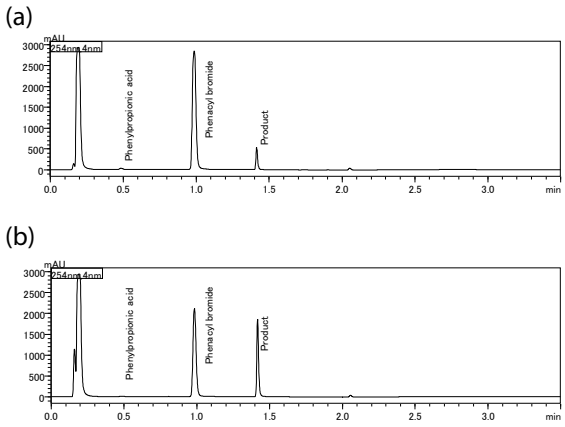

Fig. 5 Chromatograms at 15min. (a) and 300 min. (b) after adding of solution B

■ Reaction product tracking using trend plots

Trend plots, created using a multi-data report function equipped in LabSolutions for indicating the increase/decrease in the peak areas of phenylpropionic acid and the reaction product based on the analysis results of samples at 15, 30, 60, 120, 180, and 300 min, are shown in Fig. 6.

Using the multi-data report function, a report shown in Fig. 7 can be automatically created after all analyses are complete, allowing visualized confirmation of the yield and intermediates in the synthesis process.

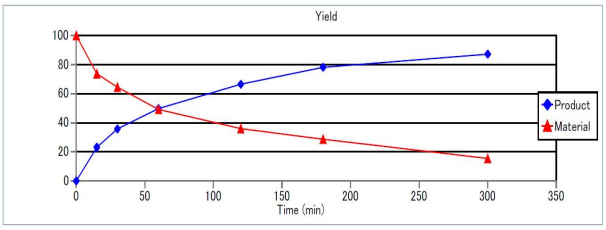

Fig. 6 Trend plot for phenylpropionic acid (reactant) and product

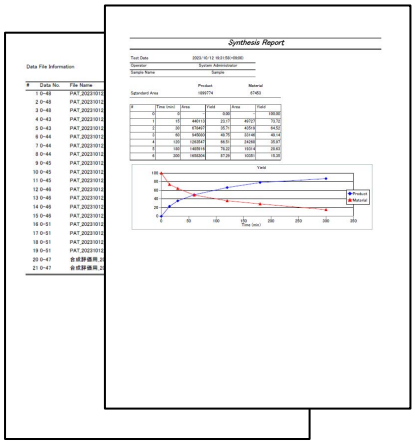

Fig. 7 Multi-data report

■ Conclusion

On-line monitoring of phenylpropionic acid labeling reaction was performed using Nexera FV. Sample aspiration using the external syringe unit provided automatic sampling and following HPLC analysis according to the analytical batch. The Nexera FV and LabSolutions FV simplified analytical batch creation, automated sampling, and automated reporting. It results in a reduction of time and increasing efficiency.

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