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Application News

High Purity Preparative Purification Enabled by UV/MS Trigger on LC-MS System

Shinichi Fujisaki, Yusuke Masuda

User Benefits

- Scaling-up from analytical column to preparative column and purity/recovery check can be completed in a single system.
- High identification performance of MS enables high purity fractionation of target compounds.
- Utilizing software simulation to optimize parameters for fractionation reduces the labor required.

■ Introduction

Preparative HPLC is utilized in various fields, such as pharmaceuticals, food, and chemical engineering, for purifying target compounds from mixed samples, searching for active ingredients in natural products, and analyzing the structures of impurities and unknown compounds. In Application News "01-00650-EN", an introduction was made to analytical/preparative convertible LC-MS system designed to manage the entire preparative purification workflow (Fig. 1). This workflow consists of the optimization of separation conditions at analytical scale, scaling-up, fractionation, and confirmation of purity/recovery. The process began with the efficient optimization of separation conditions, achieved using LabSolutions MD, a dedicated software for supporting method development, to ensure adequate separation between the target compound for fractionation (Hydrocortisone) and the nearby co-eluted peaks. Then followed by loadability optimization, scaling-up, and fractionation with UV signal as a trigger. This article introduces the achievement of higher purity in the fractionation of Hydrocortisone by effectively eliminating impurities through the use of MS signal, which offers enhanced identification performance in addition to UV signal.

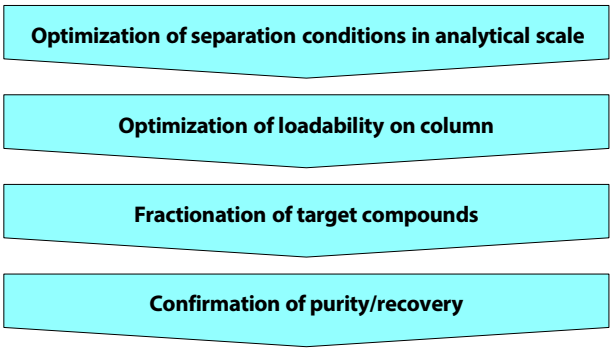


Fig. 1 Workflow of Preparative Purification

■ Overview of LC-MS System

The flow path diagram of analytical/preparative convertible LC-MS system is shown in Fig. 2. The analytical flow path (the upper of Fig. 2) is used to optimize separation conditions, loadability on column, and purity/recovery check, while the preparative flow path (the lower of Fig. 2) is used for the preparative separation of target compounds. The liquid handler (LH-40), which has both analytical and preparative flow paths and can inject fractions from fraction tubes directly into the analytical flow path, allows a complete workflow of preparative purification with this system. A combination of UV and MS signals can also be used as triggers for fractionation. Since MS detects compounds after spraying and volatilizing the mobile phase, introduced compounds cannot be recovered. Therefore, the preparative flow is split to introduce a portion of the mobile phase eluted from the preparative column into MS with make-up solvent, enabling both MS-triggered fractionation and MS detection. This system, equipped with both analytical and preparative functions, can be controlled by LabSolutions. The software features fractionation simulation

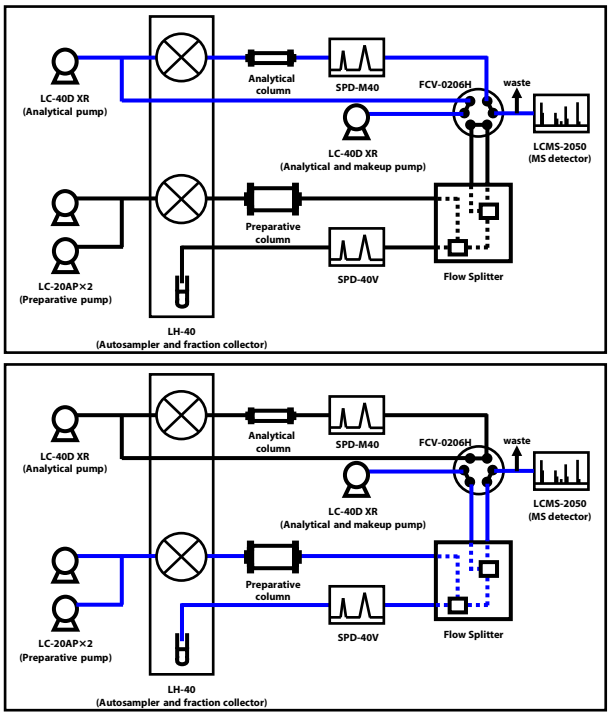
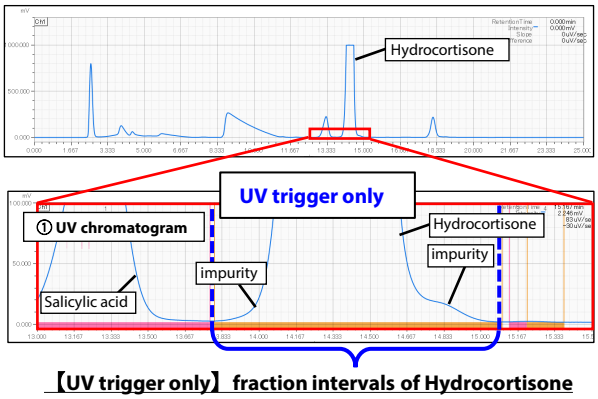


Fig. 2 Analytical Flow Path (Upper), Preparative Flow Path (Lower)
*blue colored flow path is in operation

that simplifies parameter setting for fractionation by just selecting the target peaks in the chromatogram displayed.

■ High Purity Fractionation Triggered by UV and MS Signals

Fig. 3 displays the results of fractionation simulations of Hydrocortisone using two settings: "UV trigger only" and "UV + MS triggers." In the case of fractionation using only the UV trigger (① in the upper part of Fig. 3), there was a risk to fractionate co-eluted impurities at the beginning and/or the end of the Hydrocortisone peak. On the other hand, by combining MS and UV triggers (①, ②, and ③ in the lower part of Fig. 3), impurity information from the MS chromatogram can be used for fractionation. Thus, the intervals where two impurities were eluted were eliminated from the fractionation, resulting in a higher purity of the recovered solution. The actual results of the fractionation using these settings are shown in Fig. 4 (fractionation conditions: Table 1). As demonstrated in the fractionation simulations in Fig. 3, in the case of "UV trigger only," the blue interval was fractionated, and the recovered solution contained very few impurities. However, in the case of "UV + MS triggers," the MS chromatogram of impurities (③ in Fig. 3) indicated that impurities were eluted at two locations: the beginning and the end of the Hydrocortisone peak. Consequently, the fractionation was able to eliminate these impurity intervals.



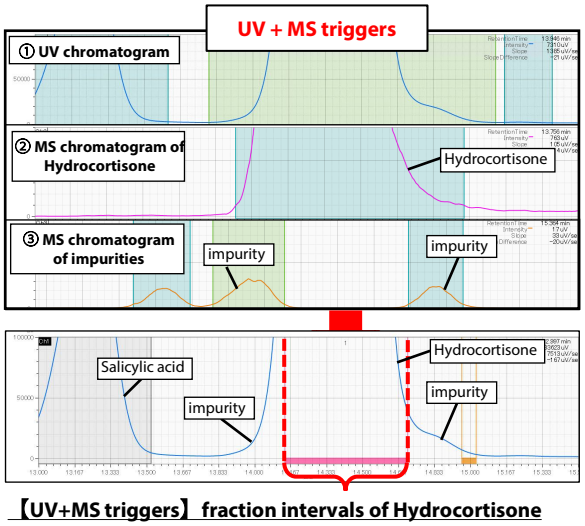


Fig. 3 Fractionation Simulation

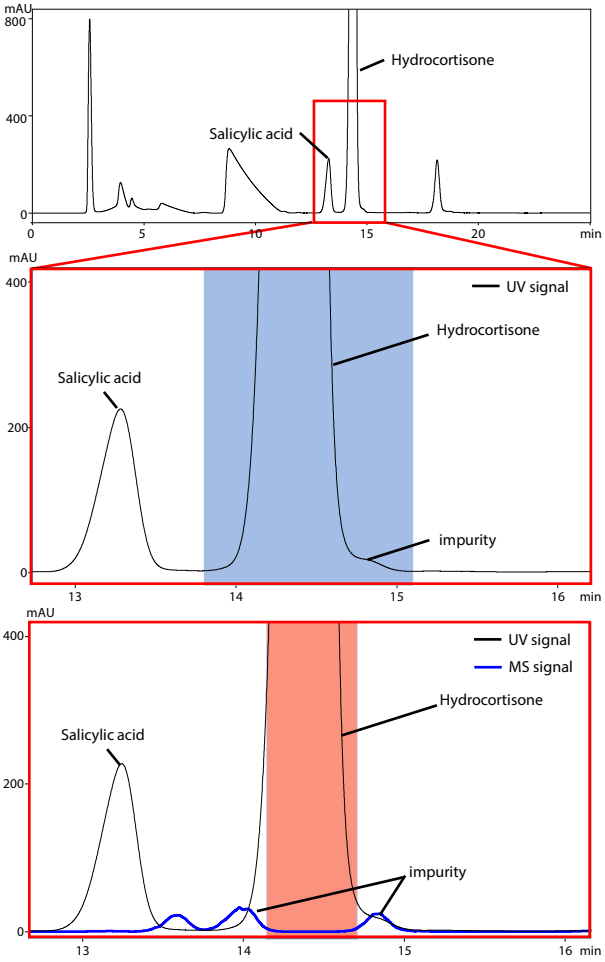


Fig. 4 Comparison of Fractionation Results between "UV trigger only" (blue colored interval) and "UV + MS triggers" (red colored interval)

Table 1 Preparative Conditions

Mobile Phase	: Pump A : 0.1% formic acid in water
	: Pump B : Acetonitrile
Column	: Shim-pack Scepter™ C18-120 (150 mm × 20 mm I.D., 5 μm)*1
Sample	: Hydrocortisone, Salicylic acid, Metoclopramide, Lidocaine, Furosemide, Papaverine, Quinidine
Sample Concentration	: 10000 mg/L (Hydrocortisone), 1000 mg/L (others)
Injection Volume	: 1 mL
LC Conditions	
Time Program	: B Conc. 25%(0 min)→45%(20 min) →25%(20.01-25 min)
Column Temp.	: ambient
Flow rate (Prep)	: 20 mL/min
Flow rate (Makeup)	: 1.5 mL/min (Methanol)
Sample loop size	: 2 mL
Syringe size : 5 mL	
Detection (PDA)	: 245 nm (SPD-40V, preparative cell)
MS conditions	
Ionization	: ESI/APCI (DUIS™), positive and negative
Mode	: SCAN (*m/z* 100-500)
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 ℃
Desolvation Temp.	: 100 ℃
Interface Voltage	: 3.0/-2.0 kV (positive/negative)

*1 P/N : 227-31102-03

■ Confirmation of Purity/Recovery

Fig. 5 shows the re-injected chromatograms of Hydrocortisone fractionated using "UV trigger only" and "UV + MS triggers" into the analytical flow path of this system, as well as the chromatogram of the mixed standard solution (used as a reference for calculating recovery). The purity and recovery rate of each fractionation are shown in Table 2. In the "UV + MS triggers" chromatogram, the re-injected chromatogram (red line in Fig. 5) indicates that no impurity peak was detected compared to the "UV trigger only" chromatogram (blue line in Fig. 5) because the target compound was fractionated in an impurity-free interval. However, since the amount of impurities was negligible, both the purity (area %) and recovery rate of the fractions obtained using "UV trigger only" and "UV + MS triggers" were close to 100%, which is a favorable result.

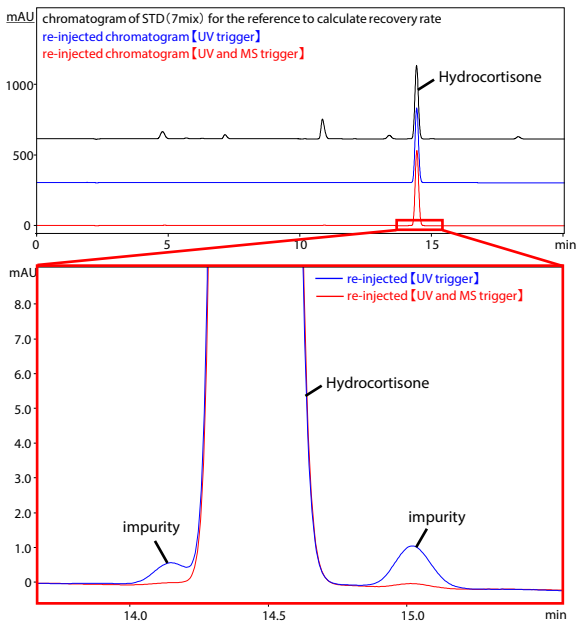


Fig .5 Chromatograms for Purity/Recovery Confirmation and STD (7mix)

Table 2 Purities and Recovery Rates of Hydrocortisone
(n=3, average value)

	Purity (Area %)	Recovery Rate(%)
UV trigger only	99.7	101.2
UV＋MS triggers	100.0	100.7

■ Conclusion

High-purity fractionation was introduced through the combined use of UV and MS signals as triggers. The utilization of an MS trigger enables the preparative purification of target compounds with enhanced purity, as it allows for the exclusion of co-eluted compounds, aside from the targets, from the fraction intervals using MS chromatogram. The analytical/preparative convertible LC-MS system employed in this article features two flow paths, one designed for analytical use and the other for preparative use, offering an efficient preparative purification workflow with this single system. For further details, please refer to Application News “01-00650-EN”.

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