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Application News

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Preparative Purification Liquid Chromatograph Nexera Prep/ELSD-LT Ⅲ/SPD-M40

Comprehensive Fractionation of Herbal Medicine Components by PDA-ELSD triggered Preparative LC

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User Benefits

- Comprehensive fractionation can be performed without missing non-UV absorbing compounds.
- Graphical user interface provides easy and labor-saving operation for fractionation settings.
- Smooth transfer from preliminary analytical LC to preparative purification LC is possible because the both LC analyses can be controlled by the same software.

■ Introduction

A preparative LC is widely known as a means of purifying target compounds from mixtures. An ultraviolet (UV) detector or a photodiode array detector (PDA) is commonly used in ordinary preparative LC. A preparative purification of all compounds without any omission is not possible because these detectors cannot detect non-UV absorbing compounds.

This article introduces a comprehensive fractionation for both UV absorbing and non-UV absorbing compounds using a newly designed PDA-ELSD triggered preparative LC that equips an evaporative light scattering detector (ELSD) in addition to the PDA.

■ Advantages of Preparative Purification by ELSD

The detectors used in ordinary preparative LC are the UV detector, which offers high versatility, and the photodiode array detector (PDA), which enables simultaneous measurements of the UV absorptions at multiple wavelengths. However, compounds that have no UV absorbing chemical structures can not be detected chromatographically because these detectors utilize the UV absorption of the target compounds. The differential refractive index (RI) detector is known as one of universal detectors that does not use the UV absorption but not suitable for the simultaneous fractionation of a wide variety of compounds due to the large variation of the baseline during gradient elution.

An evaporative light scattering detector (ELSD) measures the scattering light by the particles of the target compounds after nebulization and evaporation of the mobile phase. The target compounds can be detected regardless of their molecular structure when their volatilities are enough low. Combining the ELSD with these characteristics with a preparative LC enables an easy fractionation of some of the compounds that have not been detected.

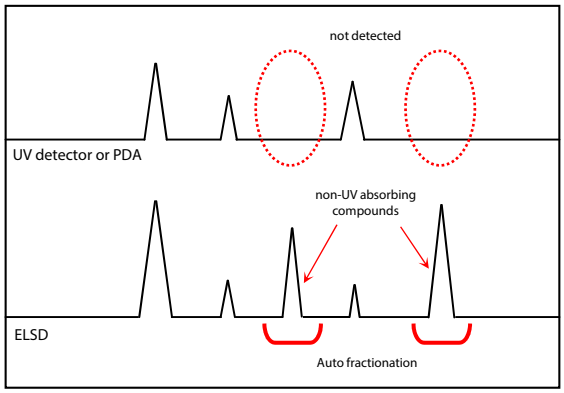

Fig. 1 Outline of Comprehensive Preparative Purification by ELSD

■ Outline of PDA-ELSD triggered Preparative LC

Fig. 2 shows the flow diagram of the system used here. The injected compounds cannot be recovered after ELSD because the mobile phase is nebulized then evaporated. This system equips a splitting flow path that distributes a large portion of eluent from the prep column to the fraction collector via the PDA and the remained small portion to ELSD for monitoring the elution behavior to send the trigger signal. Consequently most of target elution band can be fractionated without the evaporation of the mobile phase.

The Shimadzu Nexera™ Prep liquid chromatograph can be controlled from the LabSolutions™ workstation that can control the analytical LC as well. Using this software, it is possible to carry out a fractionation simulation by graphical user interface (GUI), which provides easy and labor-saving operation for fractionation settings. Fig. 3 shows the simulated fractionation of the target peaks by the auto fractionation function and corresponding real chromatograms of PDA and ELSD.

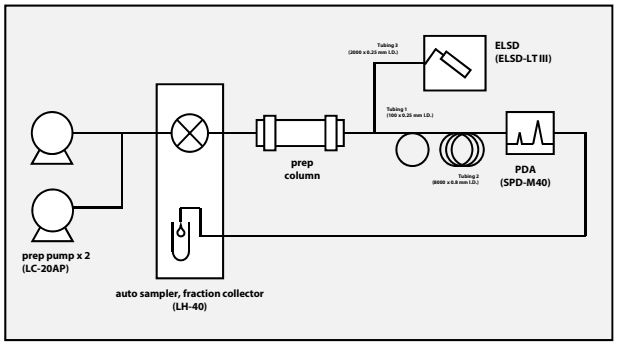

Fig. 2 Flow Channel Diagram of PDA-ELSD triggered Preparative LC

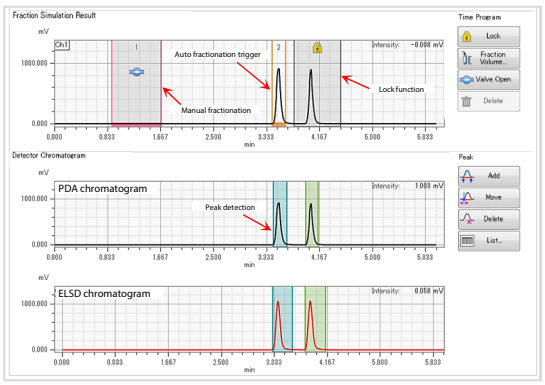

Fig. 3 Simulation of Fractionation Screen on LabSolutions

■ Optimization of Fractionation Parameters for Extract of Herbal Medicine Scutellaria Root

A herbal medicine that utilizes natural medicinal products is a general term. Most of herbal medicines are used without purification and contain many medicinal active compounds. The purification and the identification of those compounds are considered important for searching unknown pharmacologically active compounds. In this experiment, the optimization for the comprehensive fractionation of the compounds contained in scutellaria root, a herbal medicine with antibacterial activity, was carried out by analytical LC.

Fig. 4 shows the sample preparation protocol for scutellaria root powder. Table 1 shows the analytical conditions obtained as a result of the optimization, and Fig. 5 shows the results of the analysis. It was possible to separate and elute various compounds contained in the sample within the analysis time of 15 min.

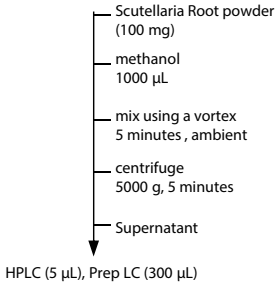

Fig. 4 Sample Preparation Protocol

Table 1 Analytical Conditions

Column	: Shim-pack™ PREP-ODS(H) Kit *1 (250 mm x 4.6 mm I.D., 5 µm)
Mobile phase	: A: water (containing 0.1 %(v:v) formic acid) B: acetonitrile/tetrahydrofuran =1:1
Flow rate	: 1 mL/min
Time program	: B conc. 25 % (0-5 min)→ 100 % (10-15 min) → 20 % (15.01-20 min)
Column temp.	: Ambient
Injection vol.	: 5 µL
Detection	: PDA; 250 nm ELSD; drift tube=40 ˚C, gain=wide, filter=2 sec

*1 S/N: 228-17881-91

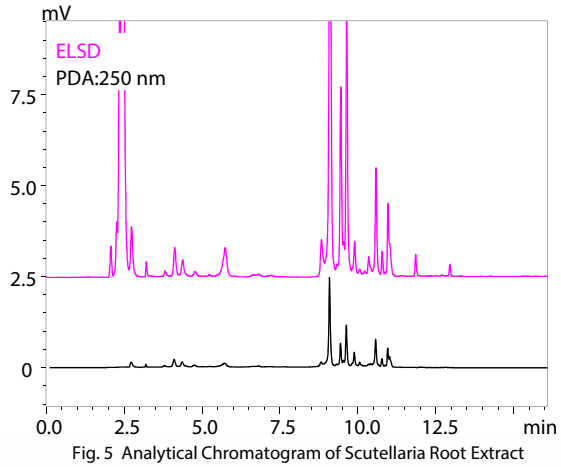

Fig. 5 Analytical Chromatogram of Scutellaria Root Extract

■ Comprehensive Fractionation for Scutellaria Root

The analytical conditions optimized by analytical LC were upscaled to the PDA-ELSD triggered preparative LC. A preparative LC method can be created based on the corresponding analytical method using this system. The PREP-ODS(H) kit affords a simple upscaling from analytical conditions to prep conditions by changing only a flow rate setting because the kit consists of both analytical and prep columns that contain identical packing materials provided from an identical manufacturing lot.

Table 2 shows the prep conditions used in the experiment, and Fig. 6 shows the obtained fractionation chromatograms. The comprehensive fractionation of non-UV absorbing saccharides and phytosterols as well as baicalin, the principal active compound contained in scutellaria root was possible by auto fractionation function.

Table 2 Prep Conditions

Column	: Shim-pack PREP-ODS(H) Kit *2 (250 mm x 20 mm I.D., 5 µm)
Mobile phase	: A: water (containing 0.1 %(v:v) formic acid) B: acetonitrile/tetrahydrofuran =1:1
Flow rate	: 20 mL/min
Time program	: B conc. 25 % (0-5 min) → 100 % (10-15 min) → 20 % (15.01-20 min)
Column temp.	: Ambient
Injection vol.	: 300 µL
Detection	: PDA; 250 nm (prep cell) ELSD; drift tube=40 ˚C, gain=3, filter=3 sec

*2 S/N: 228-17881-91

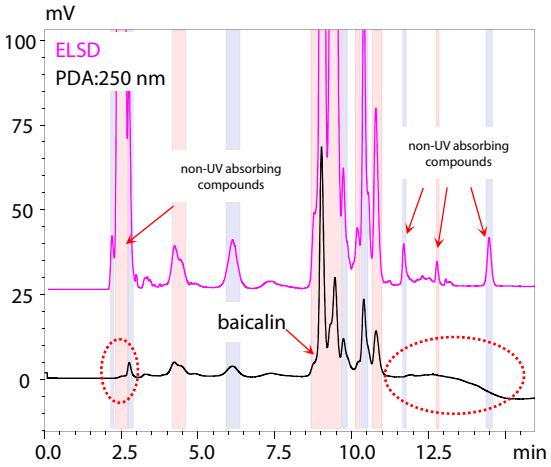

Fig. 6 Total Fractionation of Compounds Contained in Scutellaria Root Extract

■ Conclusion

This article introduced a comprehensive fractionation of the compounds contained in scutellaria root using a combination of analytical LC and PDA-ELSD triggered preparative LC. Natural products, represented by herbal medicines, contain many unknown compounds that show pharmacological activity but have not been purified and identified. The applicability of this system to the discovery and following evaluation of functionality for a unknown compound that has been difficult to purify until now is expected by creating optimized analytical LC conditions and the following comprehensive fractionation using ELSD.

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