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PO-CON1402E

High Throughput Method Scouting

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Introduction

The optimization of analysis conditions in HPLC requires considerable time and effort. Particularly with the analysis of impurities and natural substances, methods tend to be constructed via trial and error.
A search for parameters, centered on columns and mobile phases, is required. A variety of method development systems have been devised with the objective of simplifying this process. Nowadays, ultra high performance liquid chromatography (UHPLC) has become popular, due to the interest in improving productivity and lessening the burden on the environment. Method development systems utilizing UHPLC are becoming an area of interest.

System Requirements

- Scouting that can be performed quickly and easily by anyone
- Easy configuring of the mobile phases and columns required for scouting
- Easy configuring the equilibration conditions for mobile phase and column switching
- Quick creation of schedules for scouting
- Interested in investigating conditions using a variety of samples (STD, actual samples, etc.)
- Interested in easily configuring not only gradient conditions, but isocratic conditions
- Interested in reducing preparation time and eliminating configuration errors
- Interested in assessing in advance the time required for scouting, as well as the mobile phase quantities and sample quantities
- Interested in batch management of columns and mobile phases using a database
- Interested in real-time, onscreen confirmation of system status during scouting
- Interested in reducing the data analysis time
- Interested in identifying at a glance the conditions (columns, mobile phases, and gradient conditions) from the name of the data file
- Interested in arranging multiple chromatograms in a single window for comparative investigations
- Desire to see the scouting conditions reflected in the data sampled

System Configuration

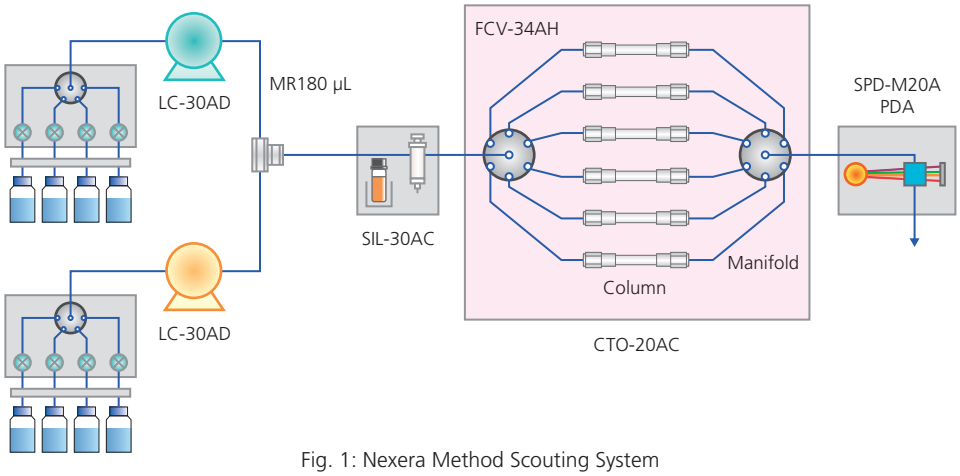


Fig. 1: Nexera Method Scouting System

- **Graphical data provided to support reliable analyses**

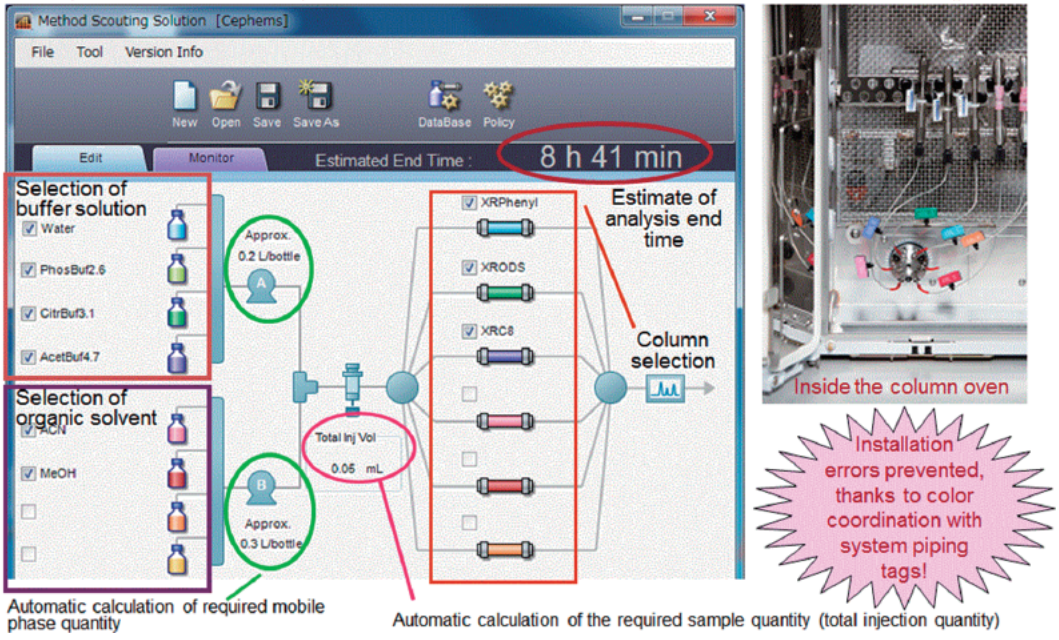


Fig. 2: Nexera Method Scouting Interface

System Capabilities

- Capable of searching conditions based on a maximum of 6 columns and up to 16 mobile phases
- Can be used with basically all current UHPLC columns (100 MPa valve pressure resistance)
- Easily configured scouting conditions thanks to special software
- Automated control of everything from system checks to scouting, and then shut down
- Quick evaluation of scouting results via data browser or Class Agent / Agent Report

Benefits

The obvious benefits of a method scouting system are time savings derived from the automated process. Columns and mobile phases to be screened are placed on the system, and the set conditions are run without human intervention over lunch, overnight, or over the weekend by automated mobile phase and column switching. This allows the method development process to be completed much sooner and frees the analyst's time for other tasks. Additionally, a significant amount of time is saved without the need to manually enter methods and individual injection conditions by using an automated method file and batch creation software.

Benefits

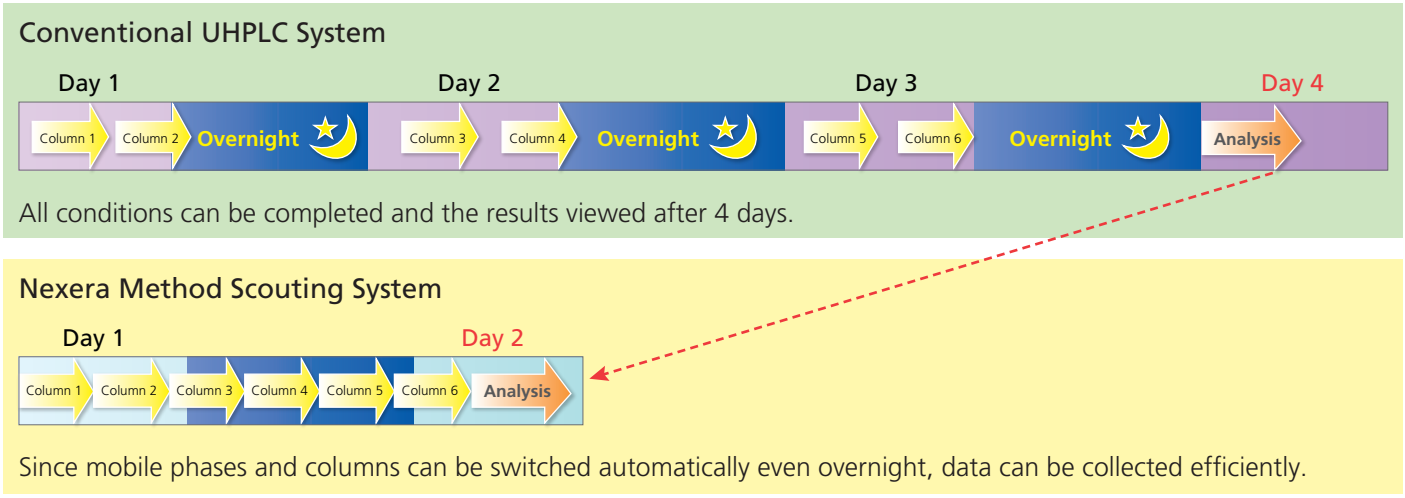
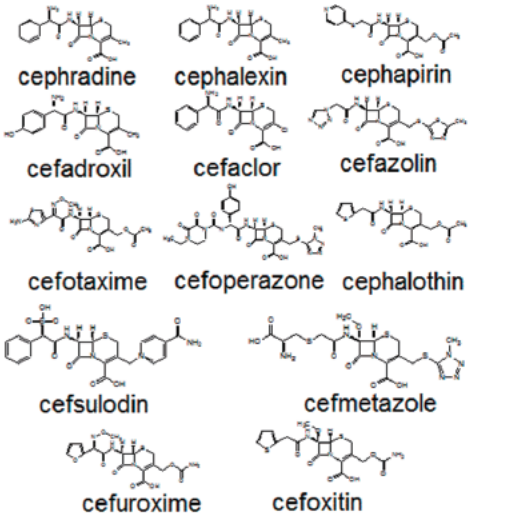


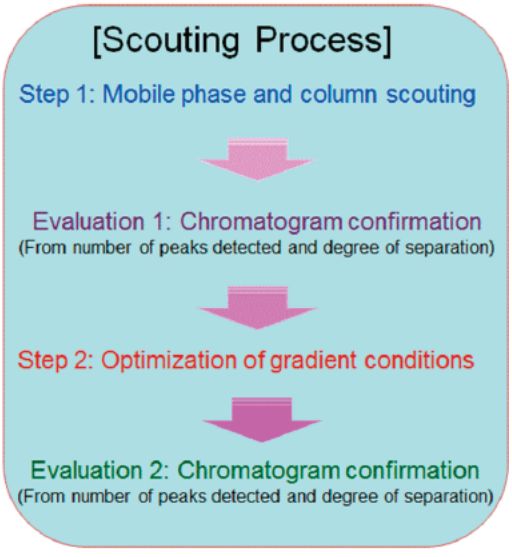
Fig. 3: Time Savings with Automated System

Automated Method Scouting of Antibiotics

Investigation of Batch Analysis Conditions for 13 Cephem Antibiotic Compounds Utilizing Nexera Method Scouting and Method Scouting Solution



Structural Formulas for 13 Cephem Antibiotic Compounds



High Throughput Method Scouting

Step 1: Mobile phase and column scouting

Purpose: To search for the optimal mobile phase and column combination with universal gradient conditions

Table 1 Step 1 Scouting Conditions

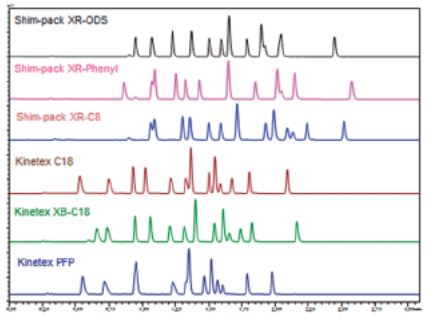
Mobile phase: (A)
- (a) Phosphoric acid (sodium) buffer solution (pH 2.6)
- (b) Citric acid (sodium) buffer solution (pH 3.1)
- (c) Acetic acid (ammonium) buffer solution (pH 4.7)
- (d) Acetic acid (ammonium) aqueous solution (pH 6.7)

Mobile phase: (B)
- (a) Acetonitrile
- (b) Methanol
- (c) Acetonitrile/methanol = 1/1 (v/v)

Column:

1) Shim-pack XR-ODS	(50 mmL. × 3.0 mmI.D., 2.2 µm)
2) Shim-pack XR-C8	(50 mmL. × 3.0 mmI.D., 2.2 µm)
3) Shim-pack XR-Phenyl	(50 mmL. × 3.0 mmI.D., 2.2 µm)
4) Kinetex C18	(50 mmL. × 3.0 mmI.D., 2.6 µm)
5) Kinetex XB-C18	(50 mmL. × 3.0 mmI.D., 2.6 µm)
6) Kinetex PFP	(50 mmL. × 3.0 mmI.D., 2.6 µm)

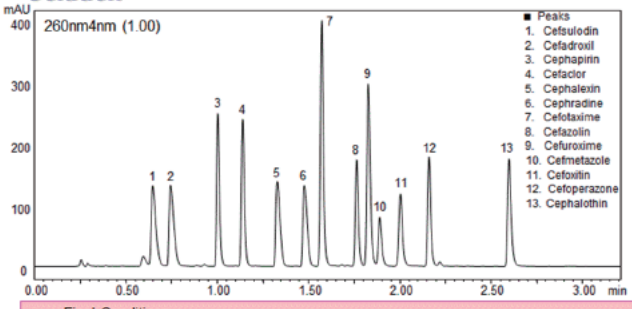
Time program: 1) B conc. 5 % (0 min) -> 90 % (5.01 min to 7 min)
Flow rate: 1.0 mL/min
Injection quantity: 5 µL
Column temperature: 40 °C
Detection wavelength: 260 nm@SPD-M20A



Evaluation 1: Chromatogram Confirmation

A total assessment is performed for the number of peaks detected, the degree of separation, and the elution time, to narrow down the columns and mobile phases suited to the analysis components.

- ***Batch analysis conditions for 13 cephem antibiotic compounds are automatically established with Nexera Method Scouting and Method Scouting Solution***



<Final Conditions>>

Mobile phase:	(A) Phosphoric acid (sodium) buffer solution (pH 2.6)
	(B) Acetonitrile
Column:	Kinetex XB C18 (50 mmL. × 3.0 mmI.D., 2.6 µm)
Time program:	B.Conc. 5 % (0 min) → 65 % (5 min) → 90 % (5.01 min to 7 min)
Flow rate:	1.0 mL/min
Injection quantity:	5 µL
Column temperature:	40 °C
Detection wavelength:	260 nm@SPD-M20A

<Time Required>

Step 1 approx. 11 hours
Step 2 approx. 4 hours

Total: approx. 15 hours

A method can be established in 1 day!

Summary

1. Automated UHPLC method scouting is an underutilized technology that greatly reduces method development times.
2. Automated scouting can free an analyst's time to perform other tasks and increase lab productivity.

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