Application Note

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The Agilent 1260 Infinity Analytical SFC System with Time-of-Flight Mass Spectrometric Detection

Method Development Using Method Scouting Wizard

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Abstract

This Application Note demonstrates the use of the Agilent ChemStation Method Scouting Wizard software for the automated development of an achiral supercritical fluid chromatographic (SFC) separation method for pharmaceuticals on the Agilent 1260 Infinity Analytical SFC system. The SFC system was equipped with a solvent selection valve and a column selection valve, enabling screening with up to 12 different solvents and six different stationary phases. The ChemStation Method Scouting Wizard software allows straightforward as well as simple method and worklist creation. This results in comprehensive and highly time-efficient method development.

Introduction

The number of applications for SFC is constantly growing. This is mainly due to the unique separation characteristics of SFC, which facilitate separation of a broad range of compounds with high efficiency[1]. Almost all achiral stationary phases known in liquid chromatography (LC) can be used in SFC, offering a broad selectivity. However, due to the compressibility of the mobile phase and the retention mechanism in SFC, which are not comparable to those known from classical LC, method development can be a challenging task. As a consequence, achiral SFC method development commonly combines screening different stationary phases with different mobile phase compositions, additives, pressures, and temperatures, which can be time-consuming. The benefits of column and mobile phase screening have been reported in an earlier Application Note[2].

This Application Note demonstrates the benefits of software-assisted method development in achiral SFC for the separation of six pharmaceutical active compounds using time-of-flight mass spectrometry (TOF MS). The method development included the screening of four stationary phases and 10 different organic solvents in the mobile phase. Flushing and equilibrations steps, as well as separation methods, were created using the ChemStation Method Scouting Wizard software.

Experimental

Instrumentation

The Agilent 1260 Infinity Analytical SFC system contained the following modules:

- Agilent 1260 Infinity SFC II Control Module (G4301A)
- Agilent 1260 Infinity SFC Binary Pump (G4302A)
- Agilent 1260 Infinity II SFC Multisampler (G4767A)
- Agilent 1260 Infinity Diode Array Detector (G1315C) with high-pressure SFC flow cell
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)

An earlier Technical Overview[3] demonstrates the performance of the Agilent 1260 Infinity II SFC Multisampler (G4767A) included in an Agilent 1260 Infinity II Analytical SFC Solution.

Additional parts required for software-assisted method development

- Agilent 1290 Infinity Valve Drive (G1170A), equipped with Agilent InfinityLab Quick Change 12-position/13-port bio-inert solvent selector valve (G4235A)
- Agilent InfinityLab Quick Change 6-position/14-port valve (p/n 5067-4142)

Mass spectrometric detection

- Agilent 6230 accurate-mass time-of-flight LC/MS (G6230B), equipped with an Agilent Jet Stream ESI source (G1958-65138)
- Agilent 1260 Infinity Isocratic Pump (G1310B) for make up flow

An earlier Technical Overview[4] demonstrates the optimization of Jet Stream ESI ionization parameters, when coupling SFC and TOF MS.

Instrumental setup

The 1260 Infinity SFC Binary Pump and the InfinityLab Quick Change 12-position/13-port valve were clustered in the Agilent OpenLab CDS ChemStation edition software. This screening used up to 12 different solvent/additive combinations, assigned in the Instrument Configuration screen of the OpenLab CDS ChemStation edition software. The 1290 Infinity Thermostatted Column Compartment was equipped with an InfinityLab Quick Change 6-position/14-port six-column selection valve, allowing screening with up to six different stationary phases. The used stationary phases were added to the column table in the Columns screen of the OpenLab CDS ChemStation. All methods were created using the ChemStation Method Scouting Wizard software (Figure 1).

The outlet of the SFC system was connected to the Jet Stream ESI source of the 6230 accurate-mass time-of-flight LC/MS. For continuous mass calibration, a 1260 Infinity Isocratic Pump was connected through a T-piece to the outlet line of the SFC. Purine and HP 921 served as reference masses for MS recalibration.

Columns

- Agilent ZORBAX Bonus-RP, 2.1 × 150 mm, 5 µm (p/n 883725-901)
- Agilent Polaris NH2, 3.0 × 100 mm, 3 µm (p/n A2014100X030)
- Agilent InfinityLab Poroshell 120 HILIC, 3.0 × 100 mm, 1.9 µm (p/n 695675-301)
- Agilent ZORBAX Rx-SIL, 3.0 × 100 mm, 1.8 µm (rapid resolution HT; p/n 828975-301)

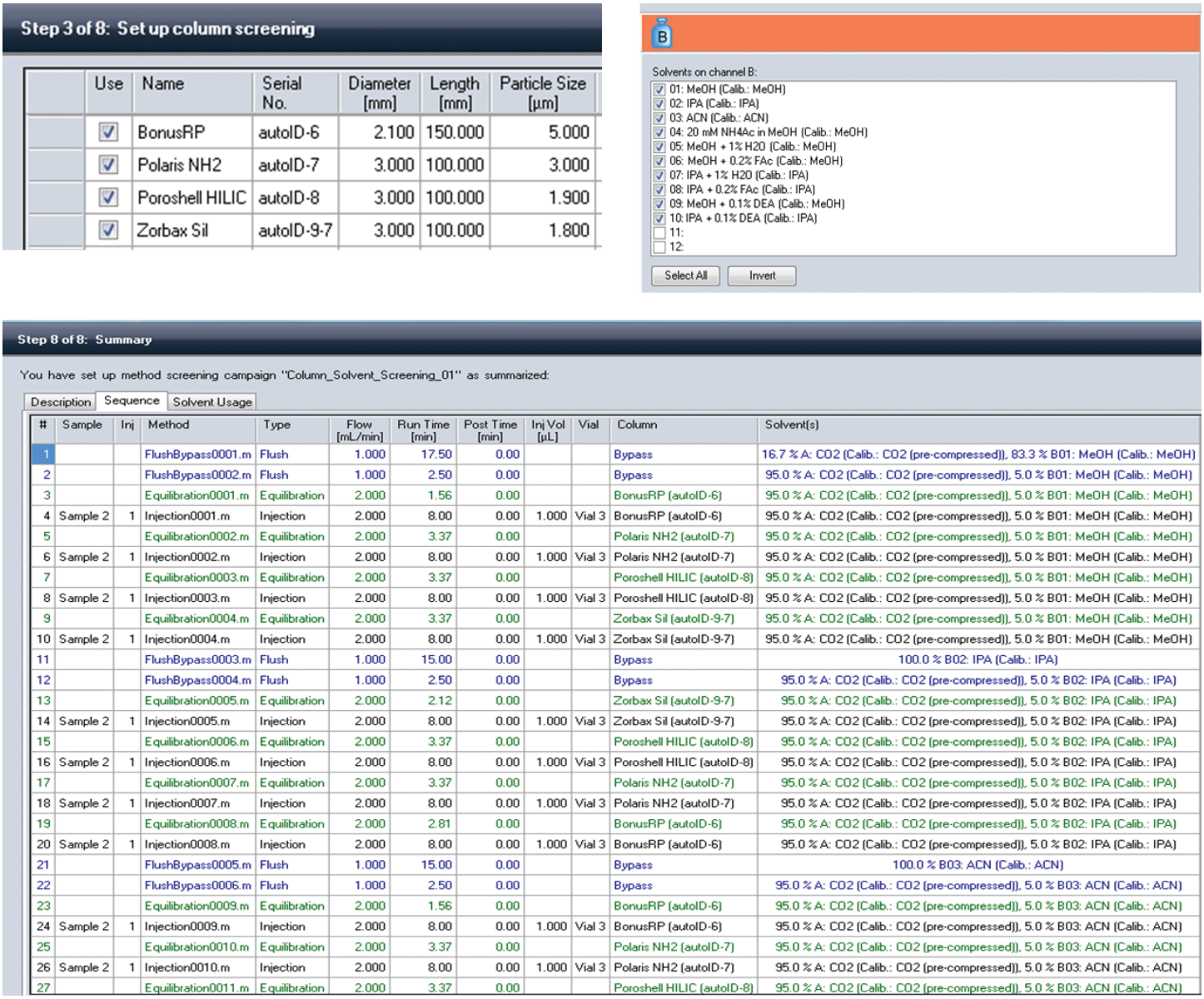

Figure 1. Setup steps of the column solvent screening, using the Method Scouting Wizard software.

Software

OpenLab CDS ChemStation Edition for LC and LC/MS Systems, version C.01.07 SR3 with ChemStation Method Scouting Wizard, version A.02.07 SR1 was used to control the SFC system and to create methods. MS data were recorded with Agilent MassHunter Data Acquisition B 05.00. For data evaluation, Agilent MassHunter Profinder version B.06.00 and Agilent MassHunter Qualitative Analysis version B06.00 SP1 were used.

Chemicals

All solvents were purchased from Merck, and all chemicals were obtained from Sigma-Aldrich. Carbon dioxide was purchased from Westfalen Gas (Muenster, Germany)

Mobile phase compositions

A: Carbon dioxide

B: Organic solvent containing the additives listed in Table 1

Results and Discussion

In the presented campaign, an SFC separation method with TOF MS detection was established for six different pharmaceutical active compounds. The aim of this study was to find a suitable combination of stationary and mobile phase, enabling the detection of all six compounds with sufficient retention resolution and peak shape. Therefore, four stationary phases and 10 different mobile phase compositions were screened with a generic mobile phase gradient method. In total, 40 different combinations of stationary and mobile phase were tested. With eight minutes run time for each combination of stationary and mobile phase and sufficient flushing and equilibration times, the whole worklist could be screened in approximately 11 hours. Data were evaluated with MassHunter Profinder, based on the sum formula of investigated compounds (batch-targeted feature extraction). For successful MS detection of compounds, signals with two or more specific ions and an accuracy of better than 10 ppm were expected. Table 2 summarizes the results of the data evaluation.

Table 1. Additives in mobile phase B.

Organic solvent	Additive
Methanol	None
Methanol	1 % water
Methanol	0.2 % formic acid
Methanol	20 mM ammonium acetate
Methanol	0.1 % diethylamine
Isopropanol	None
Isopropanol	1 % water
Isopropanol	0.2 % formic acid
Isopropanol	0.1 % diethylamine
Acetonitrile	None

Generic mobile phase gradient

Time (min)	% B
0	5
4	40
6	40
7	5
8	5

Sample

Compound	Sum formula	$[M+H]^+$	CAS-number	Compound use
Primidone	$C_{12}H_{14}N_2O_2$	219.11279	125-33-7	Anticonvulsant
Carbamazepine	$C_{15}H_{12}N_2O$	237.10223	298-46-4	Anti-epileptic
Fenofibrate	$C_{20}H_{21}ClO_4$	361.12010	49562-28-9	Antilipemic agent
Nafcillin (sodium)	$C_{21}H_{21}N_2NaO_5S$	415.13220 (no sodium)	985-16-0	*Beta*-lactam antibiotic
Decoquinate	$C_{24}H_{35}NO_5$	418.25878	18507-89-6	Antiprotozoal agent
Cloxacillin (sodium)	$C_{19}H_{17}ClN_3NaO_5S$	436.07283 (no sodium)	642-78-4	*Beta*-lactam antibiotic

Compounds were dissolved in acetonitrile and combined in a working mixture, containing 50 µmol/L of each in acetonitrile.

SFC method

Parameter	Value
Solvent A	CO_2
Modifier B	10 different combinations of organic solvent and additives
SFC flow	2 mL/min
Backpressure regulator	100 bar, 60 °C
Temperature	25 °C
Injection volume	1 µL

Mass spectrometric method (ESI positive mode)

Parameter	Value
Drying gas temperature	275 °C
Drying gas flow rate	5 L/min
Sheath gas temperature	275 °C
Sheath gas flow rate	6 L/min
Nebulizer pressure	45 psi
Capillary voltage	4,000 V
Fragmentor	150 V
Skimmer	60 V

Table 2. Retention times (RT) of compounds, screened with different combinations of stationary and mobile phases. Compounds that were not detected are marked with "–". Combinations that allowed the efficient separation and detection of all six selected compounds are marked with an asterisk (*).

Stationary phase	Mobile phase (modifier in CO_2)	Primidone RT (min)	Carbamazepine RT (min)	Cloxacillin RT (min)	Fenofibrate RT (min)	Nafcillin RT (min)	Decoquinate RT (min)
ZORBAX Bonus-RP	Methanol	0.89	0.84	1.85	0.65	–	1.59
ZORBAX Bonus-RP	Methanol + 20 mM ammonium acetate	0.86	0.79	1.54	0.63	2.10	1.52
ZORBAX Bonus-RP	Methanol + 1 % water	0.90	0.83	1.81	0.65	3.13	1.54
ZORBAX Bonus-RP	Methanol + 0.2 % formic acid	0.90	0.84	1.80	0.64	3.13	1.55
ZORBAX Bonus-RP	Methanol + 0.1 % diethylamine	–	–	–	–	–	–
ZORBAX Bonus-RP	Isopropanol	1.20	1.10	–	0.66	–	2.29
ZORBAX Bonus-RP	Isopropanol + 1 % water	1.18	1.10	–	0.64	–	2.17
ZORBAX Bonus-RP	Isopropanol + 0.2 % formic acid	1.23	1.13	2.55	0.63	3.17	2.27
ZORBAX Bonus-RP	Isopropanol + 0.1 % diethylamine	–	–	–	–	–	–
ZORBAX Bonus-RP	Acetonitrile	2.55	1.56	–	0.62	–	–
Polaris NH2	Methanol	1.80	1.45	5.56	0.73	–	2.00
Polaris NH2	Methanol + 20 mM ammonium acetate	–	1.36	–	0.65	–	–
Polaris NH2	Methanol + 1 % water	1.81	1.46	–	0.62	3.24	1.89
Polaris NH2	Methanol + 0.2 % formic acid	1.84	1.47	5.79	0.60		1.93
Polaris NH2	Methanol + 0.1 % diethylamine	–	–	–	–	–	–
Polaris NH2	Isopropanol	2.55	2.15	–	0.70	–	3.47
Polaris NH2	Isopropanol + 1 % water	2.55	2.14	–	0.67	–	3.26
Polaris NH2	Isopropanol + 0.2 % formic acid	–	2.23	–	0.68	–	7.35
Polaris NH2	Isopropanol + 0.1 % diethylamine	–	–	–	–	–	–
Polaris NH2	Acetonitrile	–	4.52	–	0.69	–	–
InfinityLab Poroshell 120 HILIC	Methanol	1.84	1.54	1.72	0.63	1.94	1.36
InfinityLab Poroshell 120 HILIC	Methanol + 20 mM ammonium acetate	–	–	–	0.60	3.63	–
*InfinityLab Poroshell 120 HILIC	Methanol + 1 % water	1.79	1.51	2.51	0.55	2.56	1.41
InfinityLab Poroshell 120 HILIC	Methanol + 0.2 % formic acid	1.83	1.53	1.60	0.61	1.79	1.38
InfinityLab Poroshell 120 HILIC	Methanol + 0.1 % diethylamine	–	1.45	–	–	–	1.39
InfinityLab Poroshell 120 HILIC	Isopropanol	–	2.18	1.53	0.56	1.70	–
InfinityLab Poroshell 120 HILIC	Isopropanol + 1 % water	2.28	2.03	1.54	0.60	1.70	–
InfinityLab Poroshell 120 HILIC	Isopropanol + 0.2 % formic acid	2.43	2.20	1.39	0.59	1.54	–
InfinityLab Poroshell 120 HILIC	Isopropanol + 0.1 % diethylamine	–	–	–	–	–	–
InfinityLab Poroshell 120 HILIC	Acetonitrile	–	4.82	–	0.71	–	–
*ZORBAX Rx-SIL	Methanol	1.95	1.65	1.44	0.58	1.73	1.73
ZORBAX Rx-SIL	Methanol + 20 mM ammonium acetate	1.95	1.64	2.99	0.53	–	1.55
*ZORBAX Rx-SIL	Methanol + 1 % water	1.95	1.63	2.44	0.56	2.62	1.53
ZORBAX Rx-SIL	Methanol + 0.2 % formic acid	1.95	1.66	1.58	0.60	1.84	1.56
ZORBAX Rx-SIL	Methanol + 0.1 % diethylamine	–	1.64	–	0.68	–	1.49
ZORBAX Rx-SIL	Isopropanol	2.49	2.28	1.55	0.64	1.81	3.97
ZORBAX Rx-SIL	Isopropanol + 1 % water	2.41	2.18	1.58	0.63	1.80	–
ZORBAX Rx-SIL	Isopropanol + 0.2 % formic acid	2.55	2.35	1.49	0.63	1.69	–
ZORBAX Rx-SIL	Isopropanol + 0.1 % diethylamine	–	–	–	–	–	–
ZORBAX Rx-SIL	Acetonitrile	–	4.85	–	0.73	–	–

Three of the tested combinations resulted in sufficient separation and detection of the six investigated compounds. These were the InfinityLab Poroshell 120 HILIC column with 1 % water in methanol, as well as the ZORBAX Rx-SIL column with pure methanol, and methanol with 1 % water. Figure 2 presents the extracted ion chromatograms (EICs) of the three different separations. It was possible to separate the six compounds in less than three minutes with all three combinations. For primidone, carbamazepine, fenofibrate, and nafcillin, sufficiently symmetric peak shapes were obtained from all separation setups. Decoquinate and cloxacillin showed peak tailing when separated on the ZORBAX Rx-SIL column using methanol. The addition of 1 % water to the modifier resulted in improved peak shape for these two compounds. The signal height of EICs was hardly influenced by the addition of water. The comparison of RTs of compounds in ZORBAX Rx-SIL separations shows that additives such as water not only impact peak shape, but can also influence compound retention (Table 3). However, these effects can hardly be predicted, and are compound dependent. For primidone and carbamazepine, retention is slightly decreased when adding 1 % water, while the retention of nafcillin and cloxacillin is significantly increased. This underlines the necessity to include different additives in the method screening procedure. These often-interacting separation-influencing factors can complicate method development in SFC.

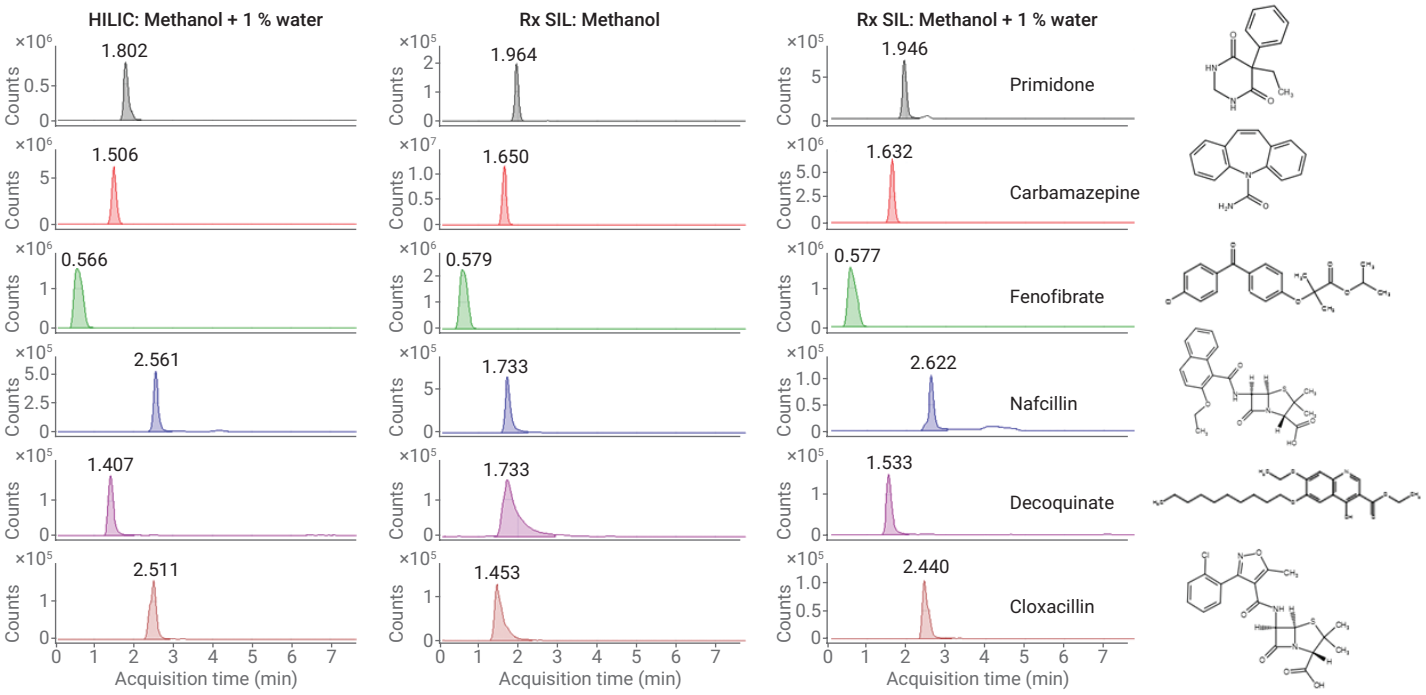

Figure 2. Extracted ion chromatograms of the six investigated compounds (20 ppm mass accuracy), separated by SFC using InfinityLab Poroshell 120 HILIC with methanol + 1 % water, Rx-SIL with methanol, and Rx-SIL with methanol + 1 % water.

Table 3. RTs and peak widths of compounds separated with ZORBAX Rx-SIL and InfinityLab Poroshell 120 HILIC using methanol or methanol + 1 % water.

Column	Modifier in CO_2	Primidone		Carbamazepine		Cloxacillin		Fenofibrate		Nafcillin		Decoquinate	
		RT (min)	Width (min)	RT (min)	Width (min)	RT (min)	Width (min)	RT (min)	Width (min)	RT (min)	Width (min)	RT (min)	Width (min)
InfinityLab Poroshell 120 HILIC	Methanol + 1 % water	1.80	0.10	1.51	0.10	2.51	0.07	0.57	0.24	2.56	0.07	1.41	0.11
ZORBAX Rx-SIL	Methanol	1.96	0.07	1.65	0.09	1.45	0.11	0.58	0.21	1.73	0.09	1.73	0.29
ZORBAX Rx-SIL	Methanol + 1 % water	1.95	0.08	1.63	0.09	2.44	0.07	0.58	0.21	2.62	0.08	1.53	0.10

Conclusion

In this study, six different pharmaceutical active compounds were separated and detected by SFC/TOF-MS. Method development considered 40 different combinations of stationary and mobile phases. Two silica phases (InfinityLab Poroshell 120 HILIC and ZORBAX Rx-SIL), both with methanol containing 1 % water (and neat for ZORBAX Rx-SIL) as modifier, were found most suitable among tested phases for the separation of the tested analytes. The Method Scouting Wizard software enables screening of multiple combinations of stationary and mobile phases in a highly automated way, and significantly reduces the time necessary to create screening procedures. It offers the option to screen and optimize separation temperature, mobile phase composition, and gradient profiles. This allows fast and comprehensive method development, considering the most important separation-influencing parameters in SFC.

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