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Excellence in Science

Nexera UC – Unified Chromatography

The latest addition to the chromatography toolbox

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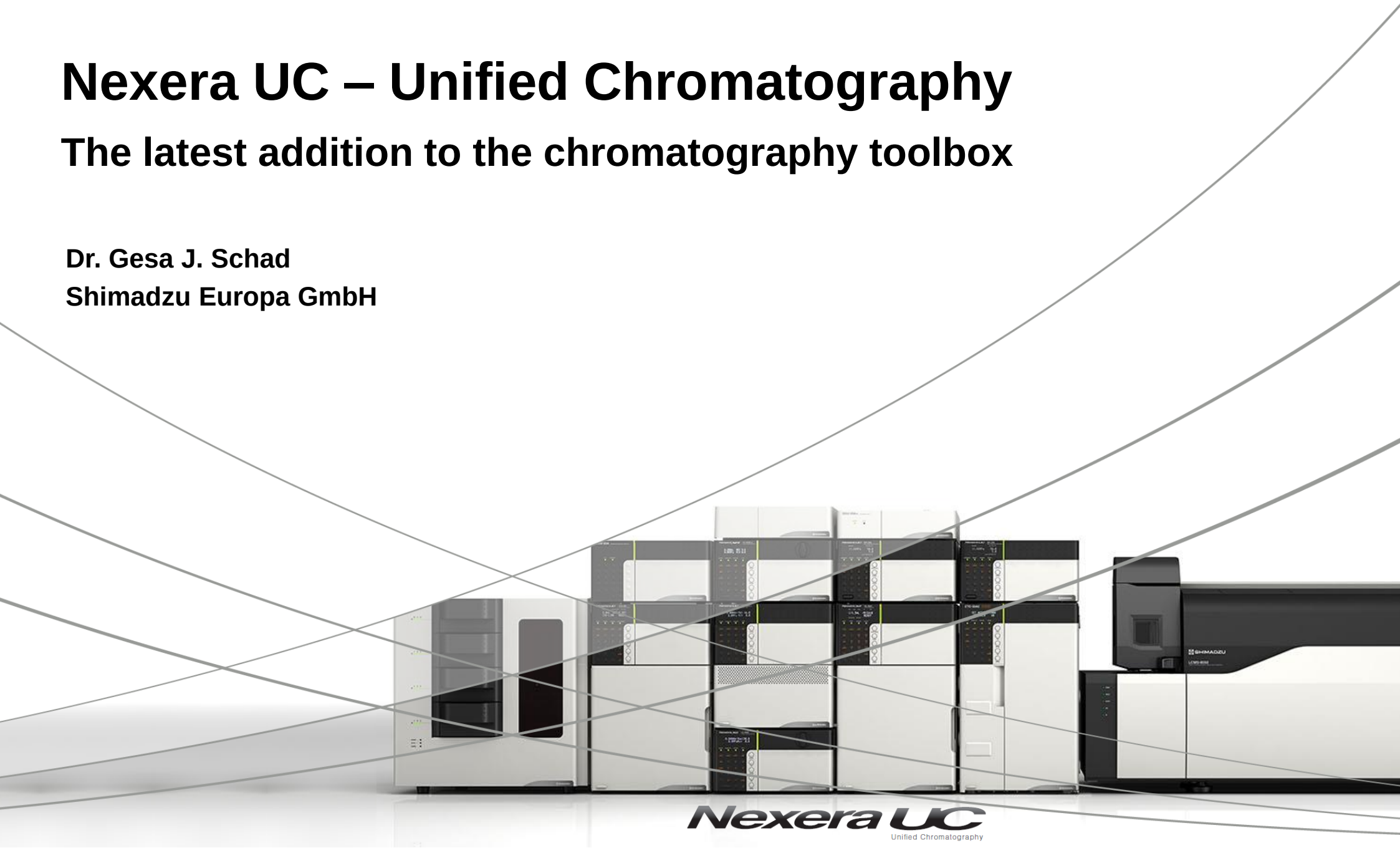
Shimadzu Europa GmbH



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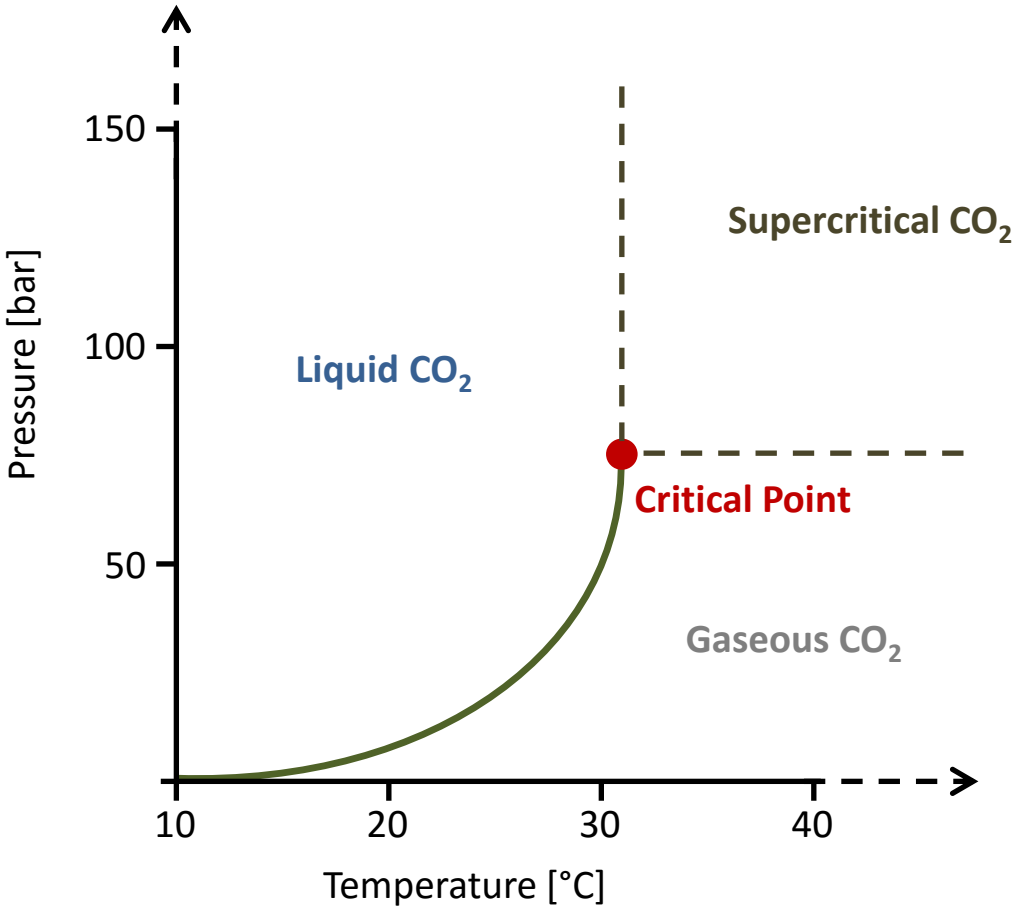
A very brief history of SFC

- Late 1800's: it was found that heavy, non-volatile organic compounds were surprisingly soluble in some inorganic gases above their critical point ('super'-critical).
- 1957: first ideas to use such gases as a chromatographic mobile phase

  → Acting as a solvent is a critical characteristic that differentiates the compressible mobile phase used in "**supercritical fluid chromatography**" (SFC) from gas chromatography (GC), where the mobile phase is considered to be an inert carrier
- Early 1990's: - concentration of polar modifiers was shown to be the primary retention control variable when added to the CO_2
  - pressure/density became a secondary control variable
  - use of additives allowed highly efficient elution of polar solutes, such as primary aliphatic amines → modern SFC was born

What is SFC and how does it work

→ Supercritical CO_2 is a fluid state of carbon dioxide where it is held at or above its critical temperature (31.1 °C) and critical pressure (73.8 bar)



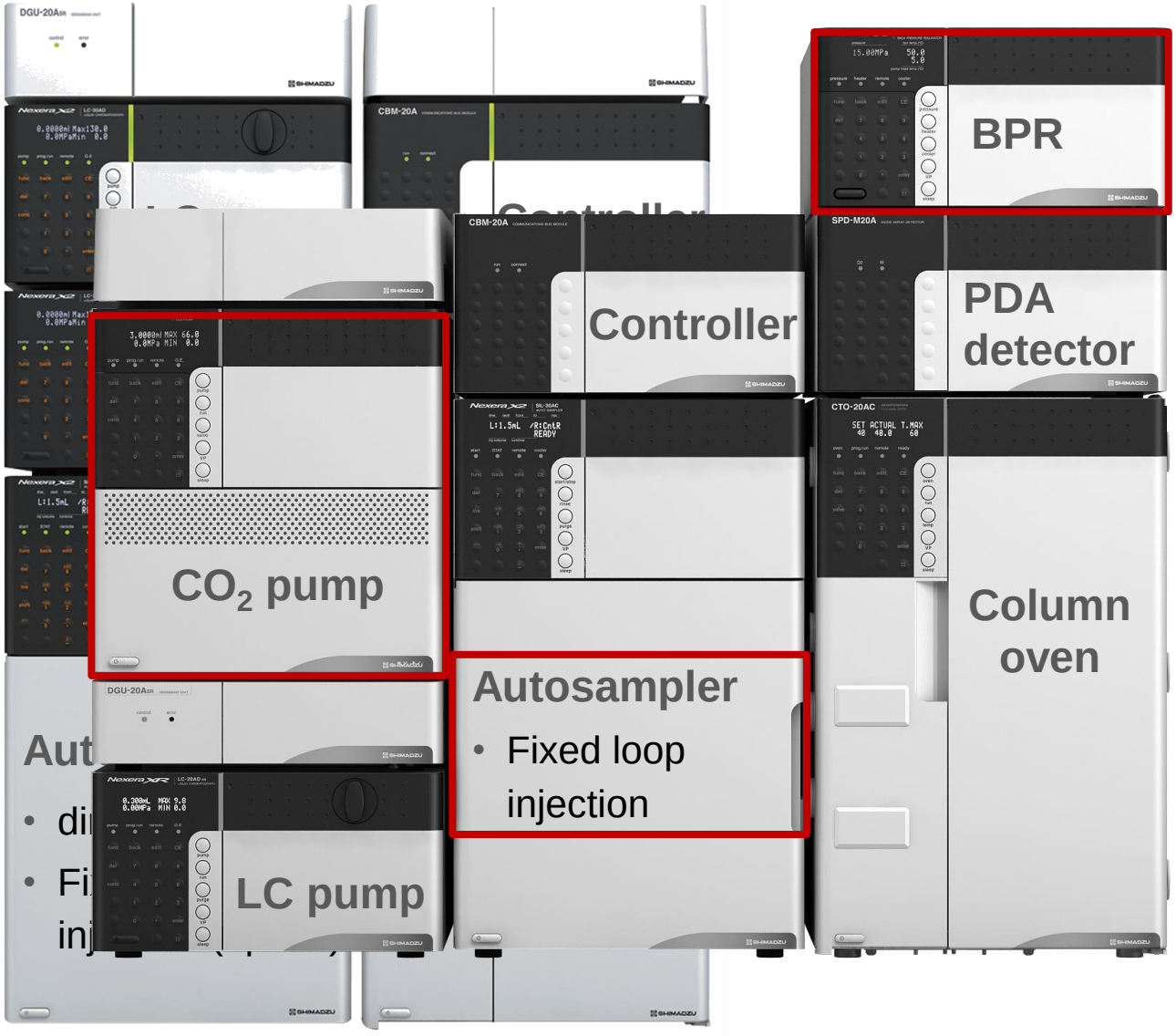
→ Rule-of-thumb: any molecule that dissolves in methanol (or less polar solvents) is compatible with SFC

→ CO_2 at its critical point is non-polar, solvent strength is increased by using a polar co-solvent

→ When a co-solvent is introduced, the mobile phase is not truly supercritical, the terminology is used regardless

SFC vs. LC

- Equipment: **HPLC / UHPLC**



SFC vs. HPLC

Similarities:

- RP-SFC, NP-SFC, IP-SFC and HILIC-SFC possible by changing polarity of stationary and mobile phase
- Organic modifiers and ionic additives can be used to adjust selectivity
- Packed column SFC is the most common methodology
- Silica based porous particles with various type of bonded phases are used for SFC separations
- Separations can be achieved isocratically or by using a solvent gradient

SFC vs. HPLC

Advantages of SFC:

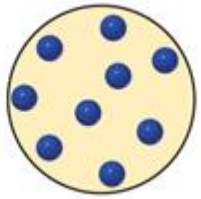
- Low viscosity of the mobile phase
  - generates up to 5 times the linear velocity of RP-LC
  - less pressure drop across the column
- Resolving power is ~ 5 x that of HPLC
- Superior to LC for chiral separations
- Complimentary chromatographic selectivity to RP-LC
- “Green” technique – reduced organic solvent consumption
- High purity samples with little residual solvent obtained in preparative chromatography

SFE – what is it and how does it work

- spCO_2 has the transport capacity of a liquid, as the molecules are pressurized to give a fluid volume
- It has the mobility and penetrating power of a gas, as the molecules are capable of behaving independently
- spCO2 can get into structures and surround molecules to be extracted using it's gas-like properties and carry those analytes out of the matrix using it's liquid – like properties.

solid

liquid



gas

supercritical fluid

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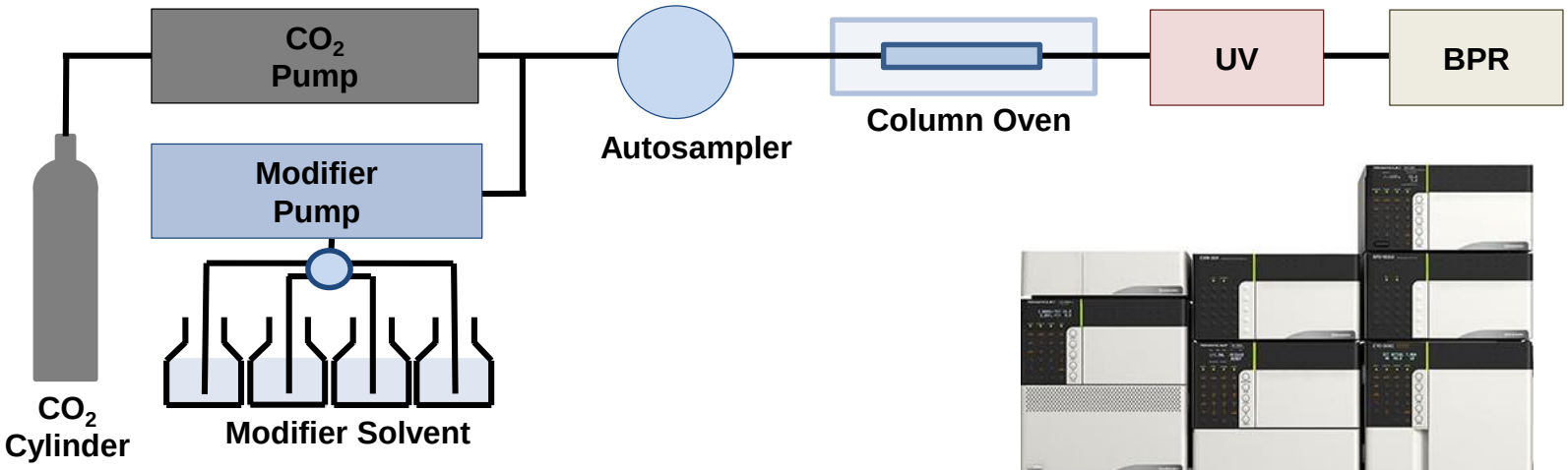
Nexera UC – Features

- Fully automated online sample preparation and analysis
  - Faster, more efficient analytical work flow
  - Reduced quantitative error
  - Less degradation of labile samples
- Multi sample extraction by automated vessel changer
  - Max. 48 sample
- Flexible use as SFC or LC system
- Improved sensitivity due to low dead volume and low pulsation BPR

Nexera UC – System Configuration

SFC / LC – UV System



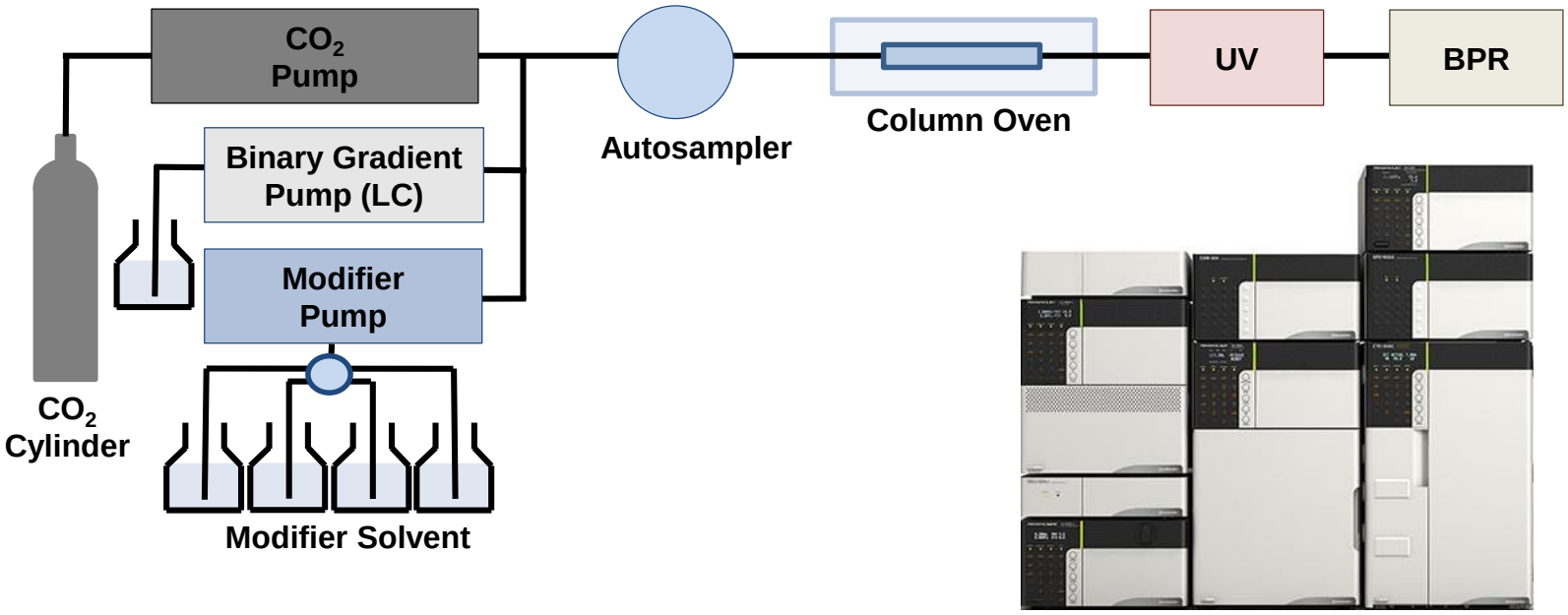
→ One Back Pressure Regulator after the detector

→ No make-up pump needed

→ Simple SFC / LC – UV hybrid system

Nexera UC – System Configuration

SFC / LC switching system – UV System



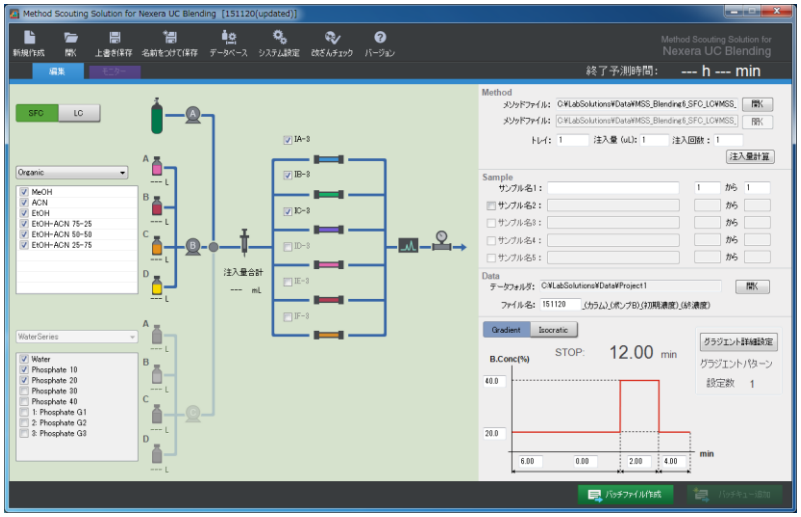
→ One Back Pressure Regulator after the detector

→ No make-up pump needed

→ Simple SFC / LC – UV hybrid system

SFC or LC analysis in one batch

Easy batch file creation for LC/SFC switching using the Method Scouting Software



分析	バイアル番号	トレイ	サンプル名	メソッドファイル	データファイル	注入量
1	1	0		0151201_SFCLC¥LCtoSFC切り替え_tr.lcm	D:¥20151201_SFCLC¥EquilibData¥2_004.lcd	1
2	-1	1		D:¥20151201_SFCLC¥SFC_base.lcm	D:¥20151201_SFCLC¥EquilibData¥2_005.lcd	1
3	1	0		D:¥20151201_SFCLC¥SFC_base.lcm	D:¥20151201_SFCLC¥EquilibData¥2_006.lcd	1
4	55	1	インダプロプライブ	D:¥20151201_SFCLC¥SFC_base.lcm	_インダプロプライブ_RP_0.1%AmAc_MeOH_5_40_012.lcd	1
5	55	1	インダプロプライブ	D:¥20151201_SFCLC¥SFC_base.lcm	_インダプロプライブ_RP_0.1%AmAc_MeOH_5_40_013.lcd	1
6	55	1	インダプロプライブ	D:¥20151201_SFCLC¥SFC_base.lcm	_インダプロプライブ_RP_0.1%AmAc_MeOH_5_40_014.lcd	1
7	55	1	インダプロプライブ	D:¥20151201_SFCLC¥SFC_base.lcm	_インダプロプライブ_RP_0.1%AmAc_MeOH_5_40_015.lcd	1
8	55	1	インダプロプライブ	D:¥20151201_SFCLC¥SFC_base.lcm	_インダプロプライブ_RP_0.1%AmAc_MeOH_5_40_016.lcd	1
9	55	1	インダプロプライブ	D:¥20151201_SFCLC¥SFC_base.lcm	_インダプロプライブ_RP_0.1%AmAc_MeOH_5_40_017.lcd	1
10	1	0		0151201_SFCLC¥SFCtoLC切り替え_tr.lcm	D:¥20151201_SFCLC¥EquilibData¥2_014.lcd	1
11	-1	1		D:¥20151201_SFCLC¥LC_Base.lcm	D:¥20151201_SFCLC¥EquilibData¥2_015.lcd	1
12	1	0		D:¥20151201_SFCLC¥LC_Base.lcm	D:¥20151201_SFCLC¥EquilibData¥2_016.lcd	1
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15	55	1	インダプロプライブ	D:¥20151201_SFCLC¥LC_Base.lcm	ンダプロプライブ_XRODS_ACN_0.1%FAw_20_90_024.lcd	1
16	55	1	インダプロプライブ	D:¥20151201_SFCLC¥LC_Base.lcm	ンダプロプライブ_XRODS_ACN_0.1%FAw_20_90_025.lcd	1
17	55	1	インダプロプライブ	D:¥20151201_SFCLC¥LC_Base.lcm	ンダプロプライブ_XRODS_ACN_0.1%FAw_20_90_026.lcd	1
18	55	1	インダプロプライブ	D:¥20151201_SFCLC¥LC_Base.lcm	ンダプロプライブ_XRODS_ACN_0.1%FAw_20_90_027.lcd	1

SFC

LC

switching method LC → SFC is automatically inserted

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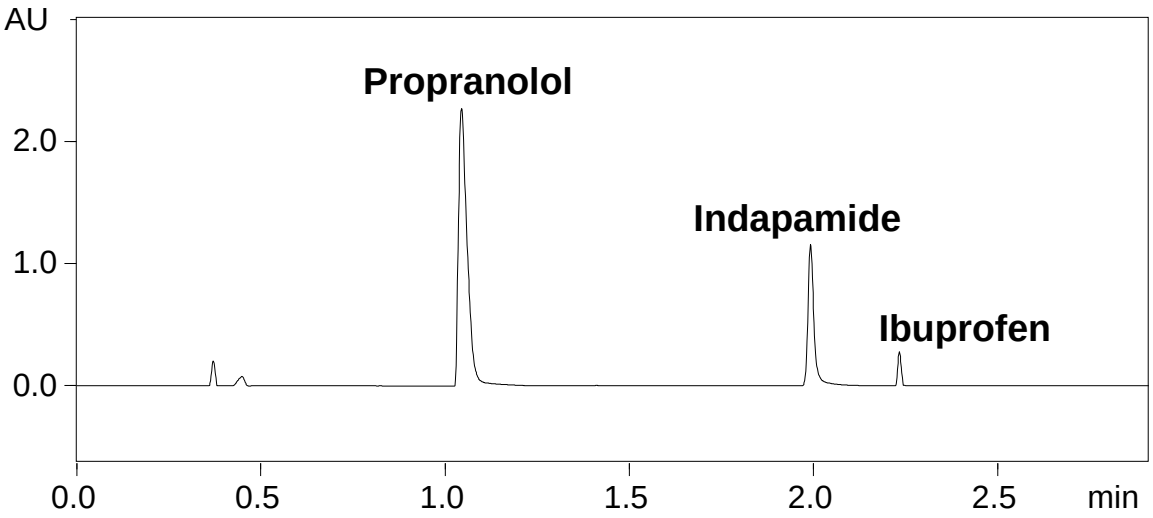
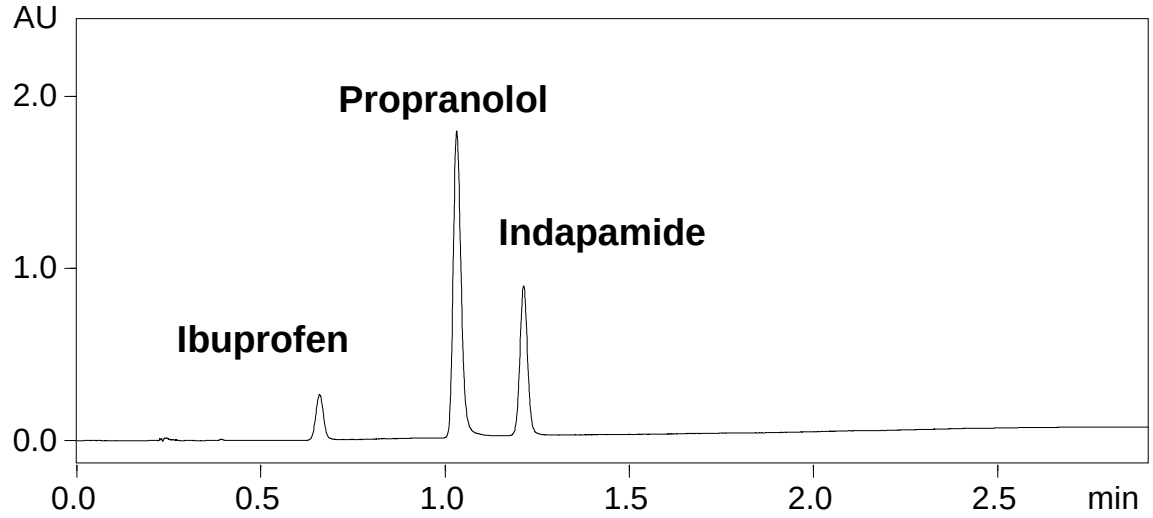
SFC or LC analysis in one batch



LC

Mobile phase A :	0.1% Formic acid in H_2O
Mobile phase B :	Acetonitrile
Flow rate:	1 mL/min
Gradient:	20 %B (0 min) – 90 %B (2-3 min)
Column:	ShimPack XR-ODS Ⅱ
	75 x 3.0 mm, 2.2 µm

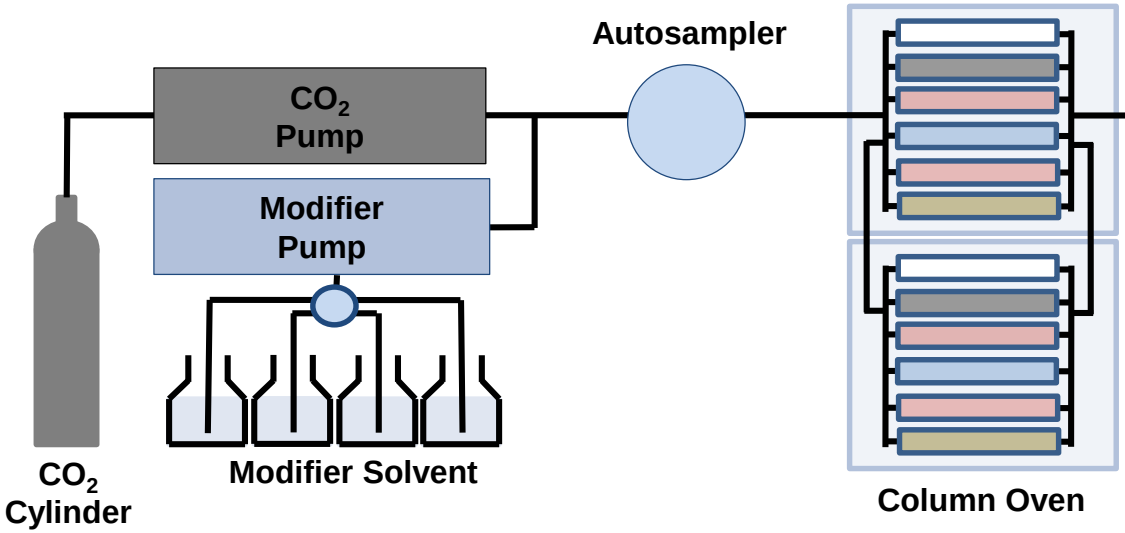


SFC

Mobile phase A:	CO_2
Mobile phase B:	0.1 % Ammonium formate in MeOH
Flow rate:	1.5 mL/min
Gradient:	5%B(0min) – 90%B(2-3min)
Column:	ShimPack UC-RP
	150 x 2.1 mm, 3 µm

Nexera UC – System Configuration

Online SFC – PDA Scouting System



→ One Back Pressure Regulator after the detector

→ Two ovens with column switching valves for fast column screening for method development

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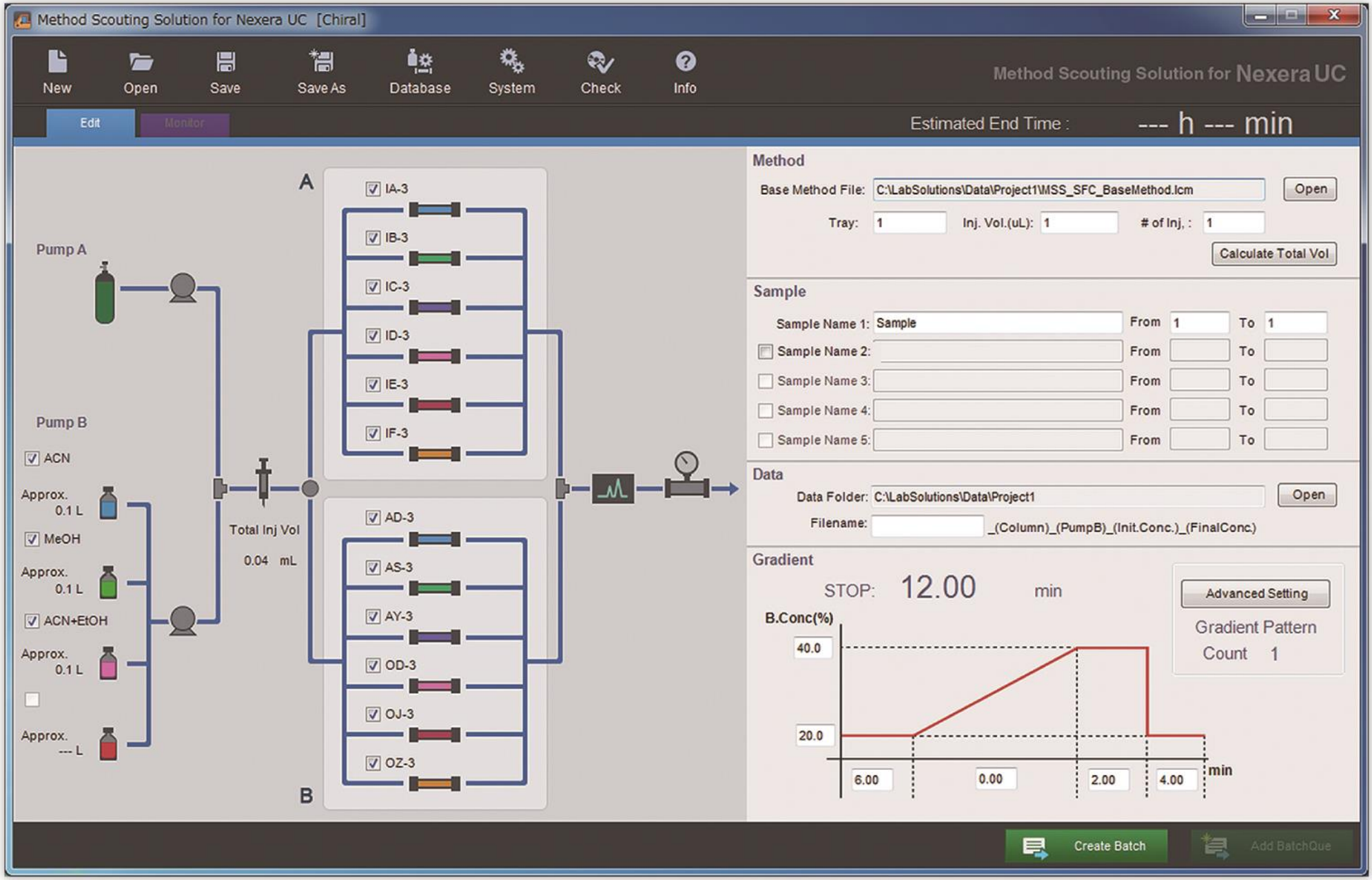
Method scouting option



Nexera UC – System Configuration

Online SFE – SFC – MS System

extraction vessel

→ Additional Back Pressure Regulator to optimize SFE conditions

→ Column switching valve for method development

→ Make-up pump for ionization for MS detection

Extraction unit

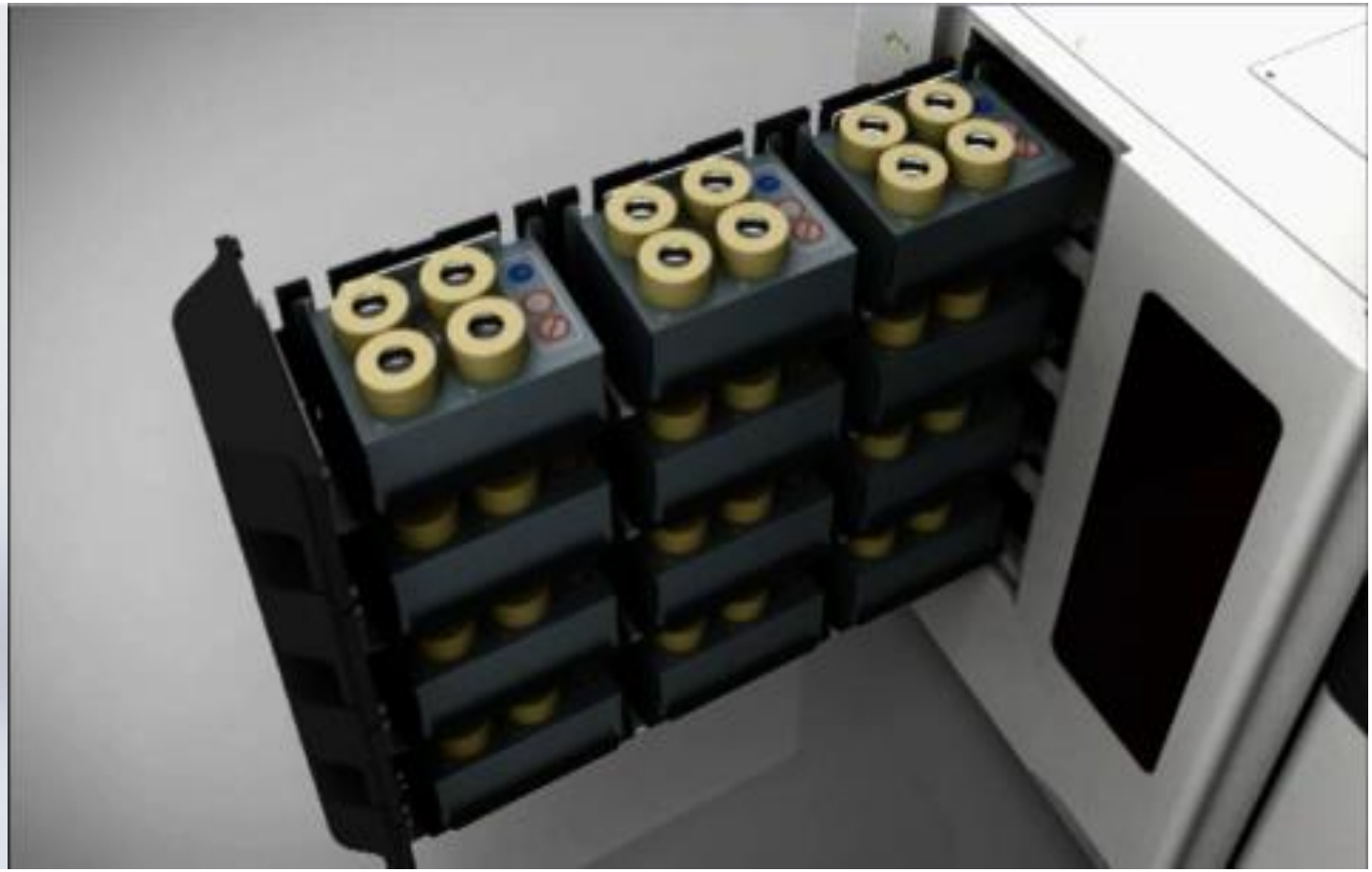
SFE-30A

Extraction up to 80ºC

Extraction vessels 0.2 mL or 5 mL

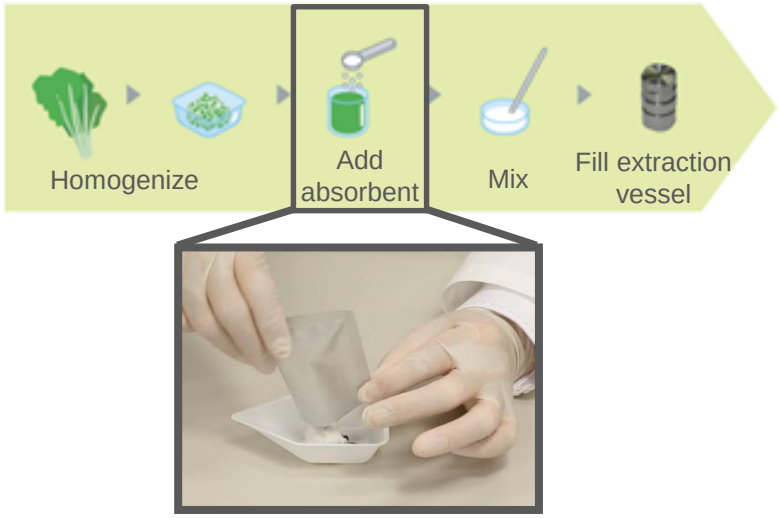
Large sample capacity

Extraction Vessel

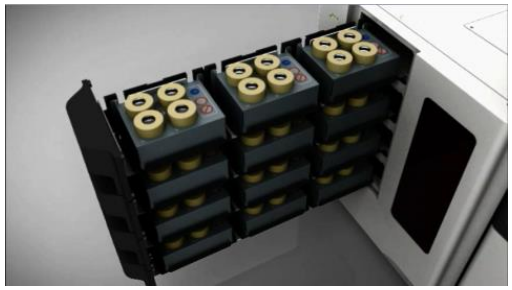


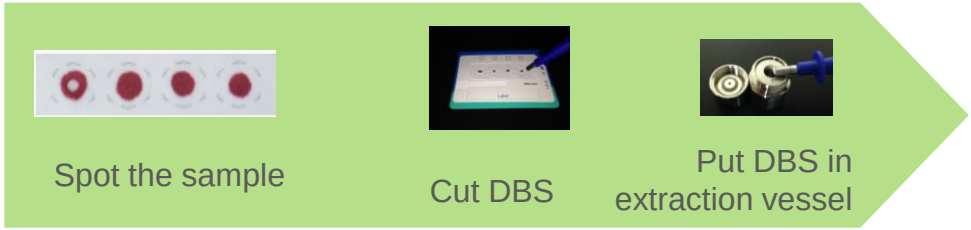
Up to 48 samples can be automatically extracted and analysed by using the Rack Changer option

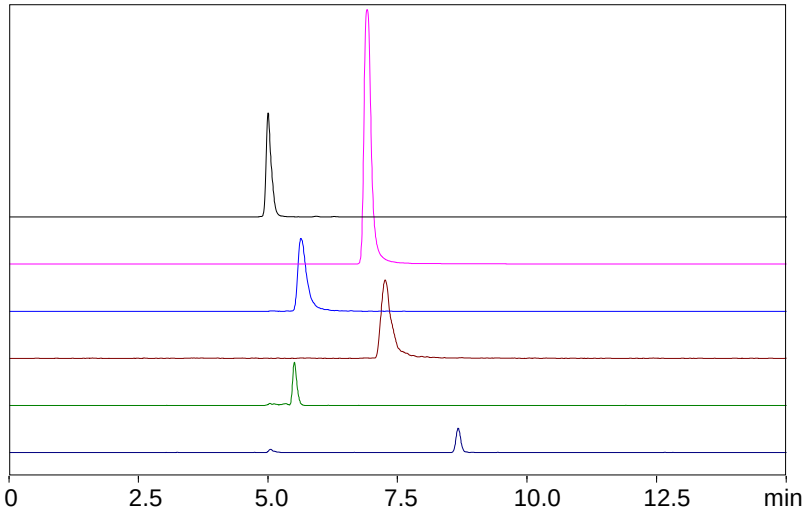
SFE sample preparation



Patented adsorbent effective for dehydration samples with high water content







Extraction and analysis of 1 ppm phospholipid spiked into plasma on DBS

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Sample preparation

Analysis of pesticides in spinach

Online SFE-SFC:

Extraction with CO_2 for 4 min (10 MPa, 40 °C)
(0 – 2 min static + 2 – 4 min dynamic extraction)

SFC Conditions:

Column: Inertsil ODS-EP HP, 4.6 × 250 mm
5 µm (GL Sciences Co.)

Modifier: 0.1% w/v (16 mM) NH_4OOCH in MeOH

Flow Rate: 3 mL/min

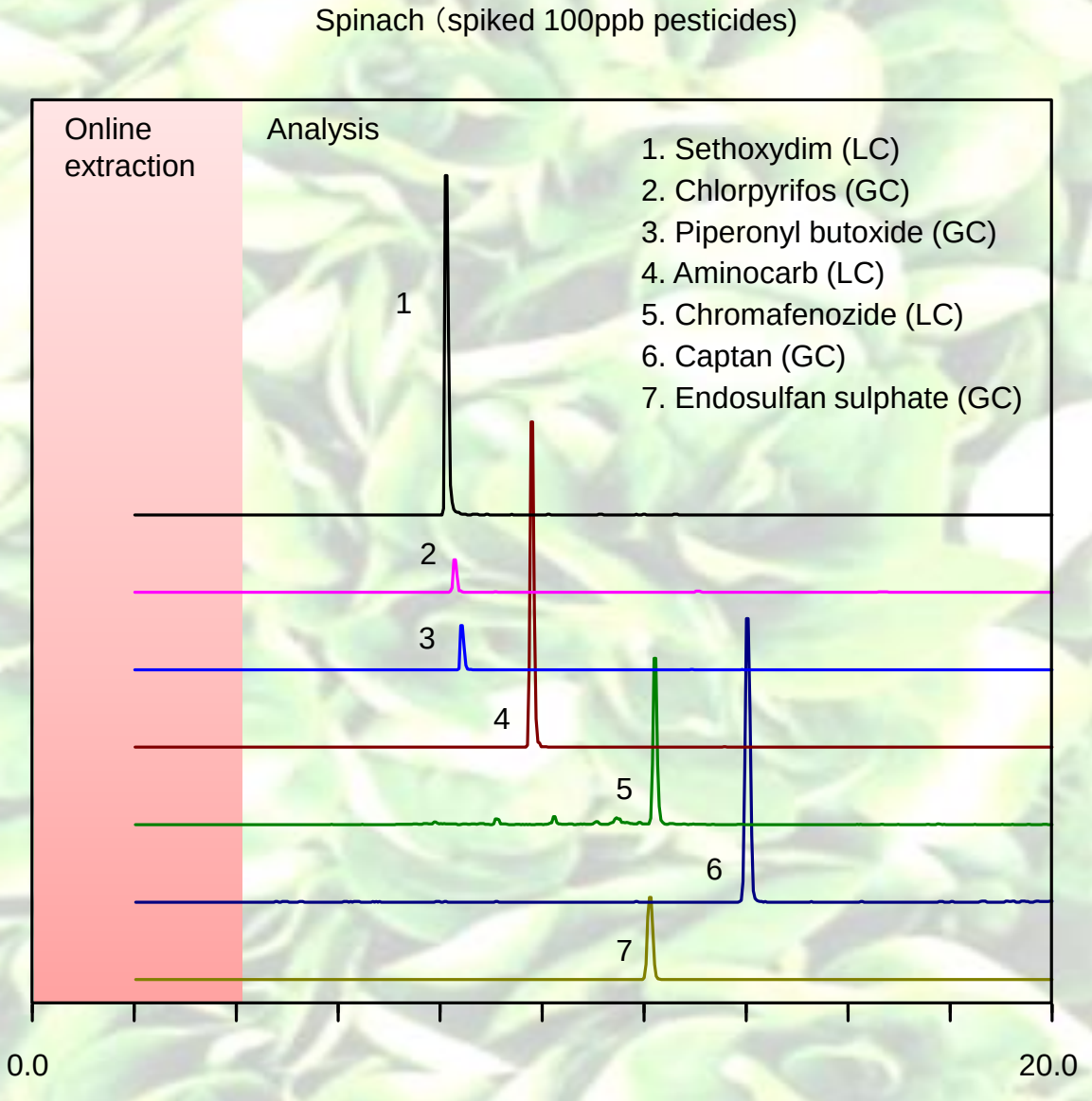
Gradient: 0 % (4 min); 11 % (15 min); 30 % (18min);
40 % (18.1 to 21 min)

BPR: 10 MPa

Detector: LCMS-8050 operating a MRM method

Make up: 0.1% w/v (16 mM) NH_4OOCH in MeOH

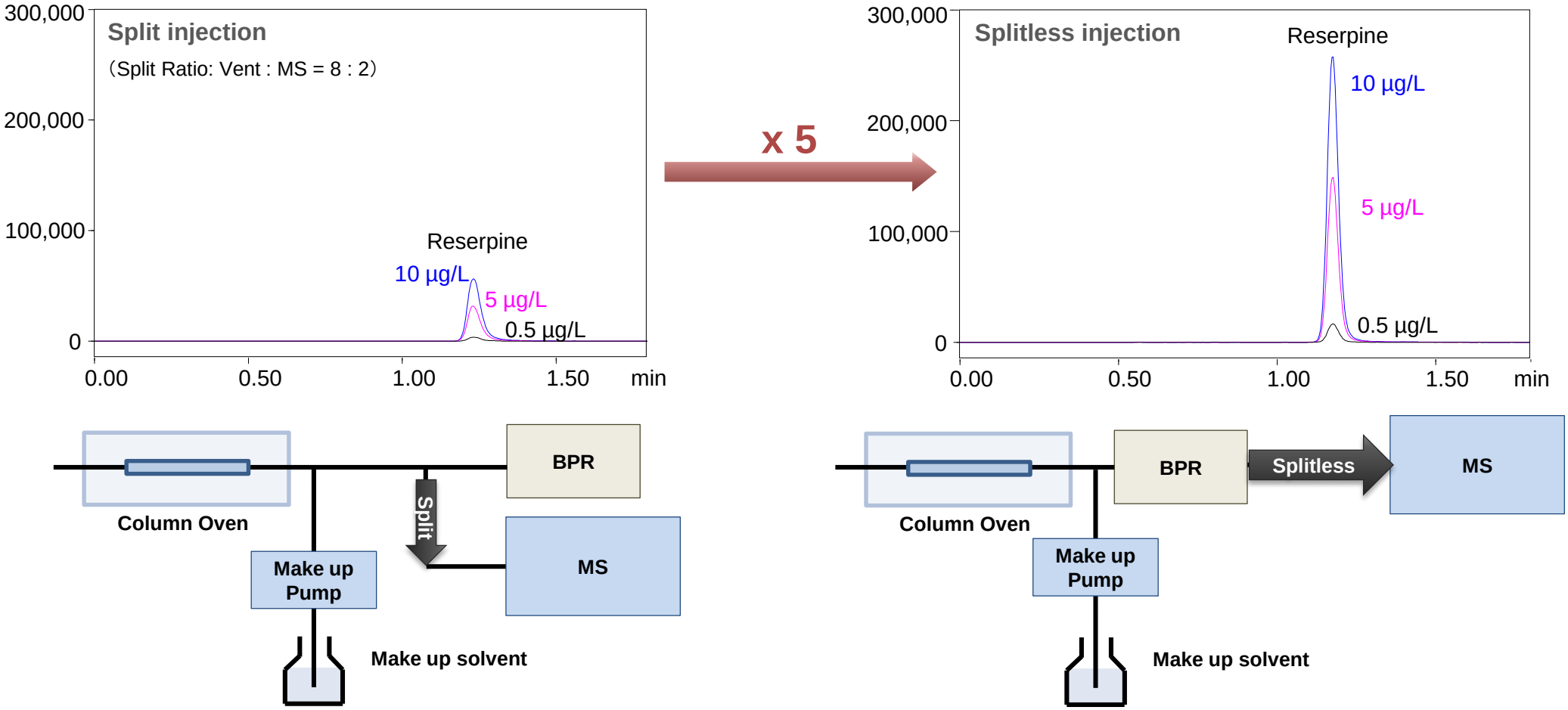
Flow Rate: 0.1 mL/min



Nexera UC – Features

→ High sensitivity detection

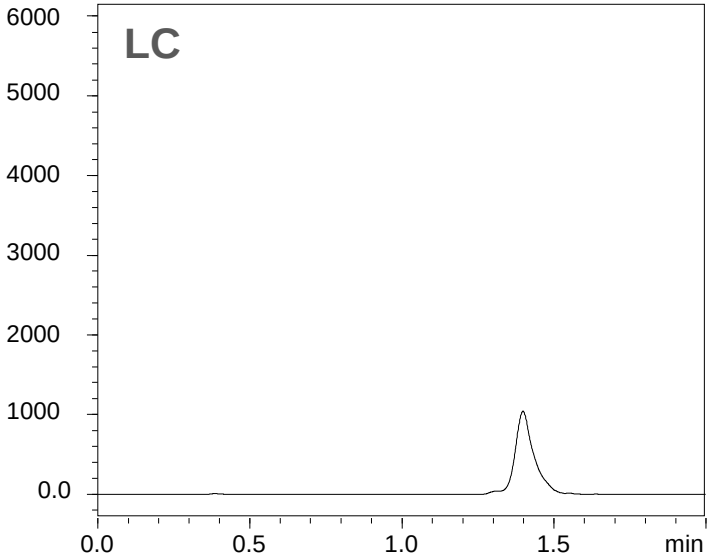
- Improved sensitivity due to low dead volume BPR
- Splitless injection into the MS without band broadening



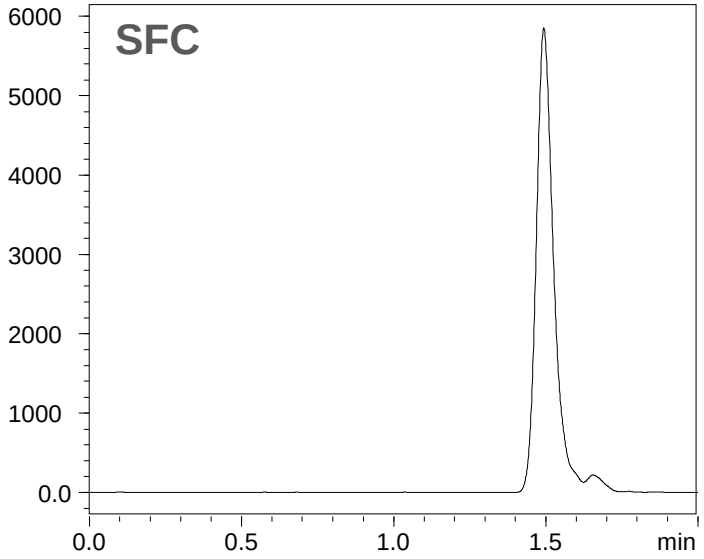
Nexera UC – Features

→ High sensitivity detection

– Concentrated sample due to evaporation of CO_2 after BPR



x 6



LC Conditions

Column	: FC ODS, 2.1×150 mm, 3um
Mobile Phase A	: 0.1% W/V Ammmonium Formate in MeOH
Mobile Phase B	: 0.1% W/V Ammmonium Formate in H_2O
B Conc.	: 30%
Flow Rate	: 0.4 mL/min
Detector	: LCMS-8040
sample	: prostaglandin 100 µg/L

SFC Conditions

Column	: Inertsil Diol, 2.1 × 150 mm, 3 m
Modifier	: 0.1 %w/v ammmonium formate in MeOH
Conc.	: 20 %
Flow Rate	: 1 mL/min
BPR	: 10 MPa (50 ℃)
Detector	: LCMS-8040
sample	: prostaglandin 100 µg/L

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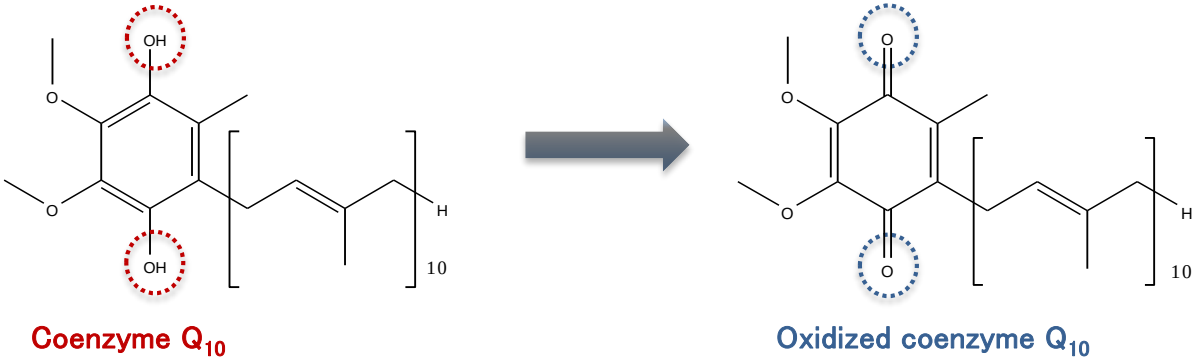
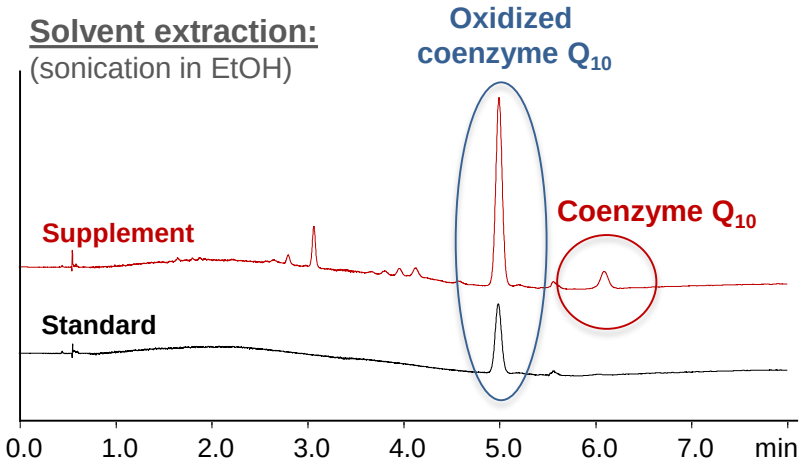
Application

Analysis of coenzyme Q_{10} in supplements

→ Conventional Method: Coenzyme Q_{10} is prone to oxidation



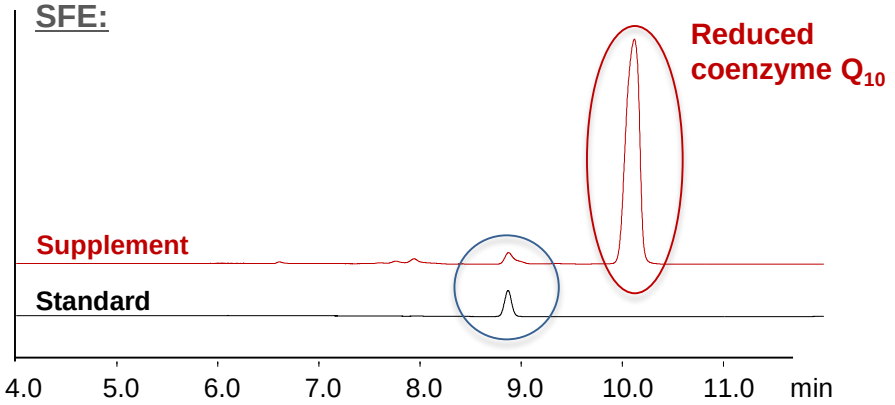
Coenzyme Q_{10} Oxidized coenzyme Q_{10}



→ No oxidation with SFE

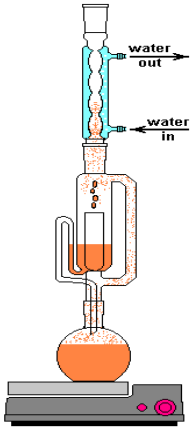
Extraction with 5 % MeOH in CO_2 for 4 min

Gradient: 5 %B (0 min – 4 min for SFE)
5 % - 50 %B in 5 min



Application

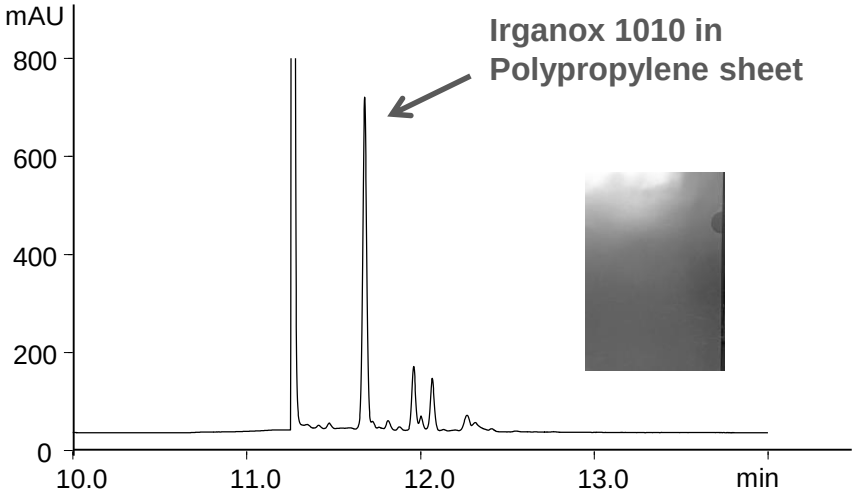
Extraction of polymer additives

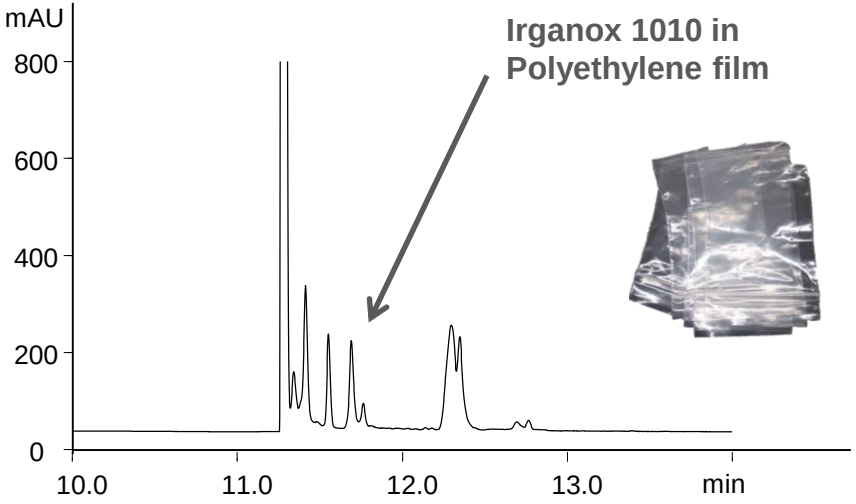


→ Conventional Method: 10 – 20 h soxhlet extraction

→ SFE: 7 min CO_2 extraction

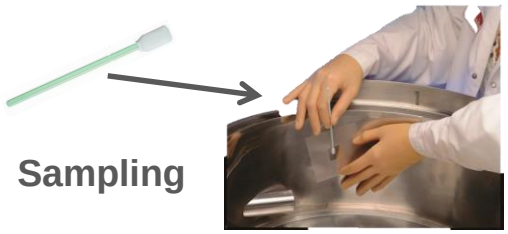
- Crushed polymer samples are filled in the extraction vessel
- After 7 min CO_2 extraction the sample is ready for SFC analysis





Application

Cleaning validation

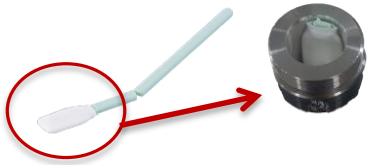


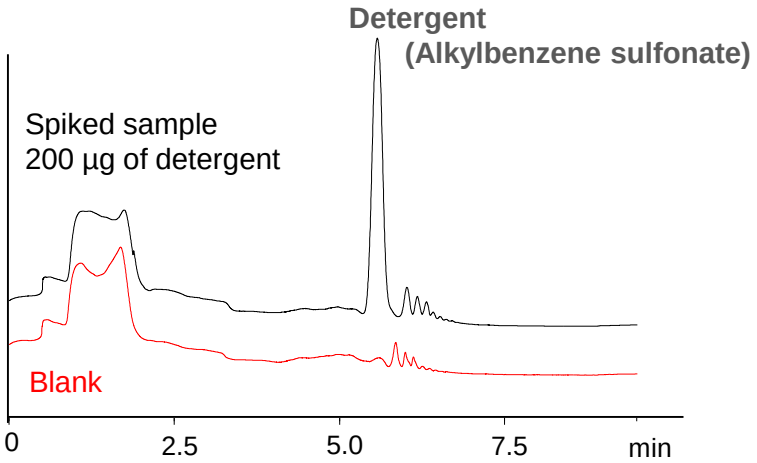
→ Conventional Method:

– Sampling – solvent extraction – concentration – HPLC analysis

→ SFE-SFC:

– Swab is enclosed in the extraction vessel for SFE



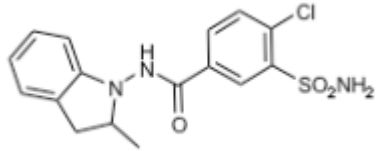


Application

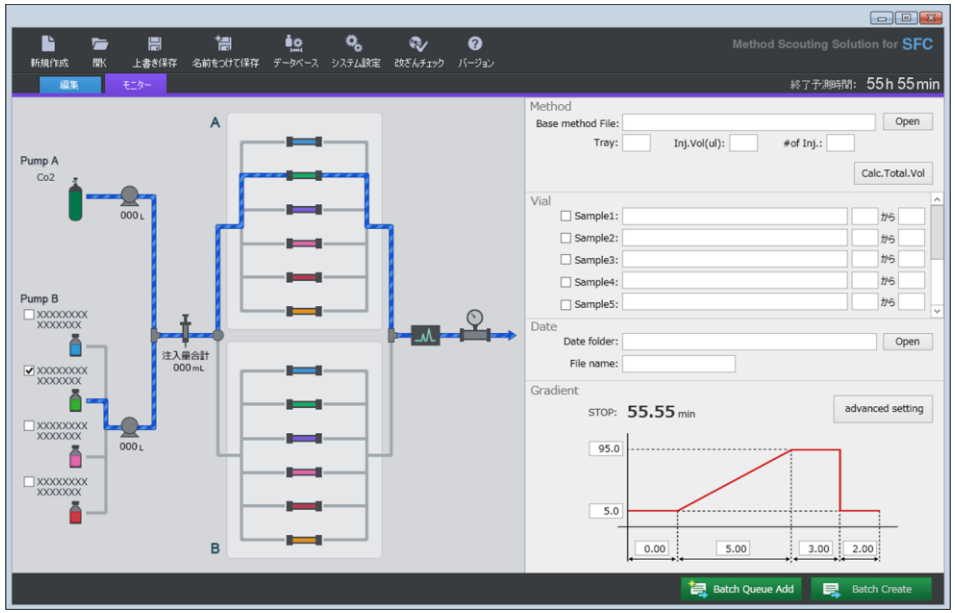
Chiral Method Scouting

→ Dedicated Method Scouting Solution:

- Selection of up to 12 columns
- Solvent blending of modifier



Indapamid



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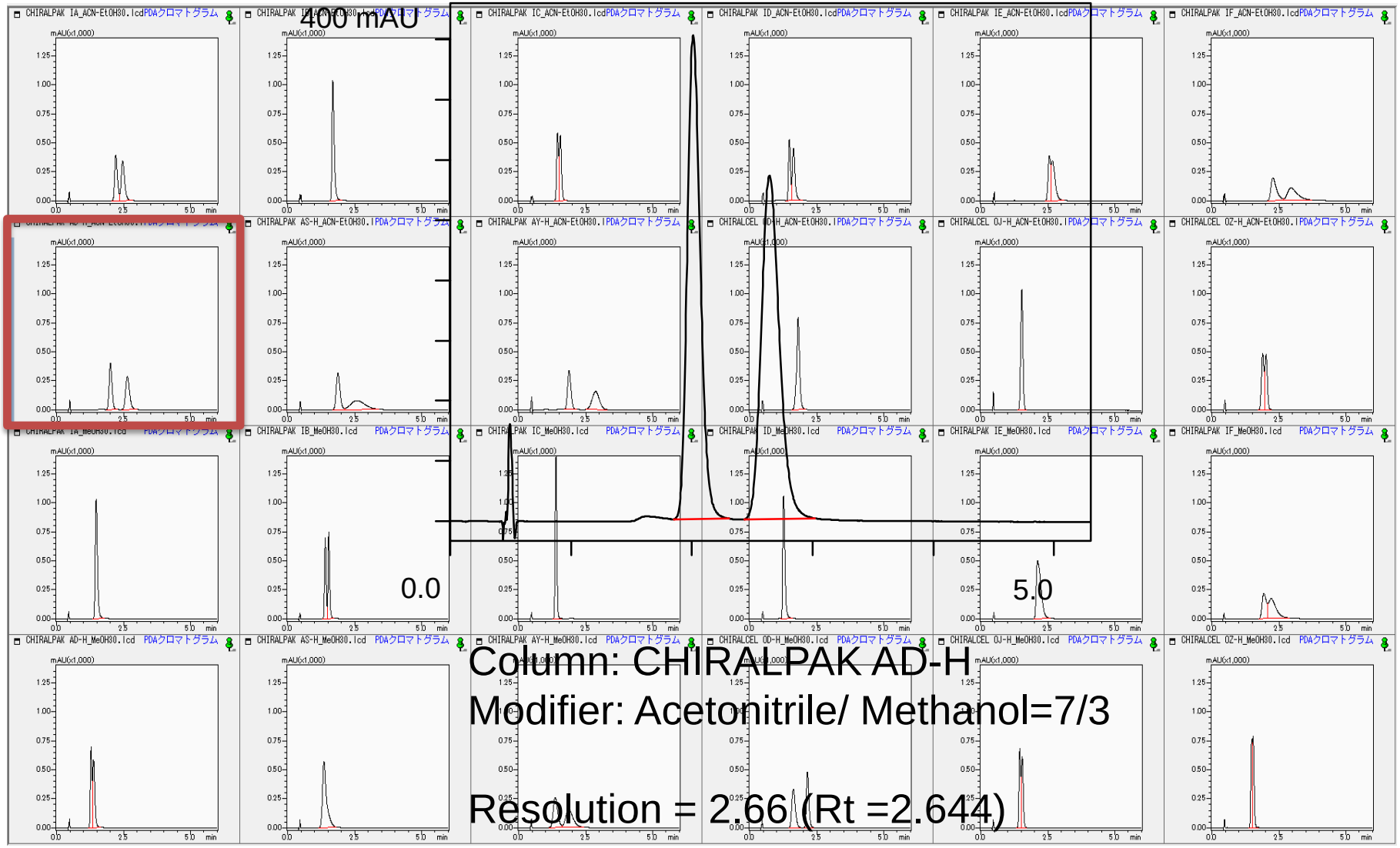
Application

→ Separation of indapamide enantiomers by method scouting



Column: CHIRALPAK AD-H

Modifier: Acetonitrile/ Methanol=7/3

Resolution = 2.66 (Rt =2.644)

Thank you for your attention !