The SFC system as a routine instrument

Danny van Oevelen
Product Specialist
Agenda

1. Introduction
2. Product
3. Applications and Application Solutions
4. Information
5. Summary
Fundamentals

SUPER-CRITICAL FLUID

NO phase transition

31.1°C
73.8 bar

SOLID
LIQUID
GAS

Courtesy of Dr. Christopher Rayner, University of Leeds
What is Supercritical Fluid Chromatography? Different Aspects

**Definition:**

1. SFC is a form of normal phase chromatography used for the analysis of low to moderate molecular weight molecules. Principles are similar to those in HPLC however SFC typically utilizes supercritical CO2 as the mobile phase; therefore the entire chromatographic flow path must be pressurized.

2. SFC can be performed at 3x higher speed compared to LC without losing separation efficiency. Solvent viscosity is lower, diffusivity higher than in LC.

3. Novel mobile phase preconditioning and superior backpressure regulation in the Agilent 1260 SFC system result in lowest UV noise and unprecedented SFC-UV sensitivity.

4. Unmatched modular flexibility and upgrade capabilities in the Agilent 1260 SFC system allow for tailored SFC systems in a broad price/performance range.
1958
SFC first proposed by James Lovelock

1962
First SFC separation of metalloporphyrins performed by Ernst Klesper (method called high pressure gas chromatography)

1983
Hewlett-Packard introduces first commercial SFC instrument

1992
Hewlett Packard re-enters SFC instrument market.

1995
Berger Instruments, founded with technology acquired from Hewlett Packard, introduces first preparative-scale SFC instruments (Berger later sold to Mettler-Toledo, then Thar Instruments, then Waters.)

2010
Agilent introduces the 1260 Infinity Analytical SFC system and the 1260 Infinity Hybrid SFC/UHPLC system, which can perform either SFC or ultrahigh performance liquid chromatography on the same instrument.
1260 Infinity and 1260 SFC Control Module
The best of both worlds

Decoupling of CO₂ compression and metering through separate pumps for highest sensitivity and reproducibility!
Agilent SFC – Wide Application Range in Many Industries

Chiral Analysis
- Chiral purity analysis (qualitative) of API
- Chiral method development for Prep SFC
- Quantitation of enantiomeric purity of starting materials, intermediates, and bulk drugs (EE)
- Metabolites, toxicology of chirals

Achiral Analysis
- Normal Phase or RP phase small molecule applications
- Drug development, library screening (lead generation)
- Pre-prep analysis, method development for Prep-SFC
- Lipids, fatty acids, vitamins
- Natural product separations
- Petrochemical, Environmental, Food and Industrial applications
<table>
<thead>
<tr>
<th>Why Are People Interested in SFC?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unique Selectivity / Orthogonal to LC</strong></td>
</tr>
</tbody>
</table>
| • Replacement for Normal Phase Chromatography  
  • Complementary to RP-LC or HILIC |
| **CO₂ Mobile Phase / Very Little Organics** |
| • High analysis speed – faster than LC!  
  • Lower operational costs and a **green technology**!!! |
| **Broad Detector Compatibility** |
| • UV, MS, ELSD… |
| **Chirals and more!** |
| • Premier choice for chiral compounds  
  • Many applications for **achiral** compounds!  
  • Polar and non-polar compounds in a single run |
| **Wide application range** |
| • LC-like robustness & sensitivity combined with SFC speed and orthogonal separation selectivity |
Fast SFC w/o losing resolution

Data courtesy: Terry Berger

250mm x 4.6mm, 3.5µ, 2.0mL/min
4 mins

150mm x 4.6mm, 3.5µ, 3.0mL/min
1.4 mins

50mm x 4.6mm, 3.5µ, 3.0mL/min
<0.65 mins

50mm x 4.6mm, 1.8µ, 5.0mL/min
<0.24 mins
High Throughput A-Chiral Separations

14 sec runs DP <200 Bar

10 chromatograms/6 minutes

4.6x50mm, 1.8µm Zorbax RX-Sil, 30% methanol at 5ml/min, 150 bar out, 50°C; ibuprofen, ketoprofen, caffeine, theophyllin, theobromine
Change in Selectivity – SFC versus rHPLC

SFC
12 overlapped injections

HPLC
10 overlapped injections

3, 4, 5, 6
Agenda

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1260 Infinity Analytical SFC Portfolio

DAD

Analytical Scale

FID

Agilent Confidential 26 Sept 2014
Agilent 1260 Infinity Analytical SFC System
Three ways to get SFC from Agilent...

- Buy SFC
  - Analytical SFC system
  - Best SFC performance

- Change from SFC/UHPLC
  - Hybrid SFC/UHPLC system
  - Orthogonal Separation Power

- Convert to SFC
  - LC upgrade to SFC system
  - Most affordable
Aurora module in detail

- Vapor Phase CO2 is delivered to the Aurora module
- Aurora module delivers preconditioned CO2 to the Agilent pump
- Aurora module delivers wash solvent
- Effluent is returned to the Aurora module for back-pressure regulation and waste collection

**Aurora module eliminates compression requirements from the HPLC pumps resulting in pulsless pump metering and low baseline noise!!!
1260 SFC Control Module

More Performance with Higher Precision

High performance pump, for auto sampler syringe flush with built-in control valve. Enables low carryover injections.

Ultrafast and High precision Back Pressure Regulator (BPR) which controls the effluent pressure downstream of the DAD.

Optimized design for CO₂ booster pump. which re-distills carbon dioxide from a gaseous CO2 supply to provide preconditioned supercritical CO2.
1260 Infinity Analytical SFC Systems

Chromatography – just better!
• SFC performance with “LC-like” sensitivity
• 600 bar x 5 mL/min power range
• 3 x faster than LC (superior diffusivity with CO₂)
• 10-15x lower operating costs with standard grade CO₂
• Low organic solvent consumption and waste generation
• Proven reliable 1200 Infinity platform
• UV, ELSD, MS

The best of two chromatographic technologies in a unique system
• Orthogonal method screening in one single system
• Direct results comparison between SFC and LC
• No instrument to instrument variation
• Efficient instrument utilization through unique capability to switch between SFC and UHPLC within a single sequence
• No equilibration time between LC and SFC
• Significant cost saving, only one system has to be purchased, saves lab space
Agilent 1260 Infinity Analytical SFC
Flow path
Agenda

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Why is orthogonal sample information important?

- Separate and detect impurities which co-elute when using a single technique
- Resolve peaks of complex samples
- Provide/get stronger evidence for sample identity
- Cover a broader polarity range of samples
Agilent 1260 Infinity Hybrid SFC/UHPLC System
Two, orthogonal techniques on a single system

PAHs with the Hybrid SFC/UHPLC System

**LC-mode**
Column: Zorbax Eclipse PAH (4.6x 150 mm, 5 um)
Mobile phase A: H2O, B: ACN

**SFC-mode**
Column: Zorbax Eclipse XDB C18 (4.6x 150 mm, 5 um)
Mobile phase A: CO2, B: MetOH, 2% H2O
Advantages of the Agilent Hybrid SFC/UHPLC instrument

• Orthogonal method screening in one single system
• Switch from SFC to UHPLC forth and back in a single sequence
• No equilibration time between LC and SFC
• Significant cost saving, only one system has to be purchased
• Direct results comparison between SFC and LC
• No instrument to instrument variation
• Save lab space
• The only vendor who can offer both techniques in one system
1) Single software control of state-of-the-art SFC now on all Agilent LC/MS platforms!

2) SFC/MS allows fast, high resolution separations of compounds which cannot easily be separated by LC methods, with limited use of organic solvents - green technology

3) Agilent analytical SFC/MS systems provide high flexibility, high precision, and greatest reliability
1260 Infinity SFC with MS Detection
Single software control of SFC on all current Agilent LC/MS systems

- Full integration of SFC and MS provides reliable instrumentation for method development and routine analysis
  - MS detection increases peak capacity and provides compound identification
  - Accurate mass screening with Q-TOF
  - Trace level quantitation with QQQ
  - AJS-ESI and APCI sources
- Several configurations
- Two different MS inlet modes supported
  - Full flow
  - Split flow
Two Ways to Connect to the MS

**Split Flow**
Only a small portion of the effluent goes to the MS

- High Resolution
- Qualitative Analyses
- Screening and Identification
- MS Passive Splitter Kit

**Full Flow**
All effluent goes through the BPR into the MS

- High Sensitivity
- All molecules make it into the source
- Good for quantitative analyses
Connection of the SFC to other detectors

The splitter assembly

- Connecting the splitter assembly

![Diagram of the splitter assembly](image)

- From make-up pump
- To BPR
- 50 µm x 100 cm Capillary to ELSD or MS
SFC Tab in MassHunter
Applications
Can SFC separate my components?

✓ Any solute **soluble in methanol** or a less polar organic solvent will elute in SFC (>80% of small drug molecules)
✓ Strong organic acids and bases, require a modifier and an additive in the mobile phase
✓ Most salts of **organic** acids and bases elute
✓ Smallish peptides elute
✓ Solutes with mass <25,000 can be separated with carbon dioxide based fluids

✗ Solutes requiring an aqueous environment
✗ Solutes requiring a buffered or ionic aqueous environment, i.e. biomolecules such as proteins will NOT elute (maybe)
✗ Most **inorganic** salts
✗ Solutes with mass >25,000 mw will not elute with carbon dioxide
Comparison of SFC vs. HPLC chiral Separation of Naragenin

SFC: 3 Minutes

HPLC: 6.5 Minutes

October 12, 2015
Fish Oil Analysis by SFC/QTOF
Fish Oil Analysis by SFC/QTOF: Extracted Ion Chromatograms

DGs

Vit D precursors

TGs

2beta-methyl-1beta,25 dihydroxycholecalciferol

Commercial fish oil capsule
Reproducibility: Retention Time and Area
Fish oil sample, EIC 561.448 and 1039.8695, 3 replicates, MS QTOF data

<table>
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<th>m/z</th>
<th>RT Area</th>
<th>Mean</th>
<th>S. Dev.</th>
<th>% S. Dev.</th>
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<th>m/z</th>
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<td>7.016</td>
<td>578.67</td>
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<td>0.0173</td>
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</table>

0 mins 3% MeOH/5mM NH₄OAc
5.5 mins 3% MeOH/5mM NH₄OAc
11 mins 60% MeOH/5mM NH₄OAc (hold for 1 minute)

0 mins 3% MeOH/5mM NH₄OAc
5.5 mins 3% MeOH/5mM NH₄OAc
11 mins 60% MeOH/5mM NH₄OAc (hold for 1 minute)
Analysis of vegetable oils and antioxidants

Which tocopherols are present?
This Application describes the analysis of 14 antioxidants in vegetable oils using the Agilent 1260 Infinity Hybrid SFC/UHPLC system in the LC and LC-MS mode.

Since the biological activities and chemical properties of tocols (tocopherols and tocotrienols) differ from each other, it is important to be able to determine each vitamer separately.

The complete resolution of the eight tocols is only possible by using the SFC-MS mode.

In addition in this case the separation by SFC was significantly faster than with UHPLC.
Antioxidants in Vegetable Oils

Chromatogram

<table>
<thead>
<tr>
<th>No</th>
<th>Name</th>
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<tr>
<td>1</td>
<td>Propyl Gallate (PG)</td>
</tr>
<tr>
<td>2</td>
<td>Tert-butyl-hydroquinone (TBHQ)</td>
</tr>
<tr>
<td>3</td>
<td>6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (TROLOX)</td>
</tr>
<tr>
<td>4</td>
<td>Butylated hydroxyanisole (BHA)</td>
</tr>
<tr>
<td>5</td>
<td>Octyl Gallate (OG)</td>
</tr>
<tr>
<td>6</td>
<td>Butylated hydroxytoluene (BHT)</td>
</tr>
<tr>
<td>7</td>
<td>Lauryl Gallate (LG)</td>
</tr>
<tr>
<td>8</td>
<td>δ-Tocotrienol (δ-TT)</td>
</tr>
<tr>
<td>9</td>
<td>γ-Tocotrienol (γ-TT)</td>
</tr>
<tr>
<td>10</td>
<td>α-Tocotrienol (α-TT)</td>
</tr>
<tr>
<td>11</td>
<td>δ-Tocopherol (δ-TP)</td>
</tr>
<tr>
<td>12</td>
<td>γ-Tocopherol (γ-TP), β-Tocopherol (β-TP)</td>
</tr>
<tr>
<td>13</td>
<td>α-Tocopherol (α-TP)</td>
</tr>
</tbody>
</table>

Column: Poroshell 120 EC-C18, 2.1 x 100 mm, 2.7 µm
Mobile phase A: A = Water + 0.1 % TFA
Mobile phase B: Methanol + 0.1 % TFA
Gradient: at 0 min 20%B, at 15 min 100%B, at 25 min 100%B
Flow rate: 0.4 mL/min
Col. temp.: 30 °C
Inj. volume: 15 µL
Data acq. (DAD): 292/10 nm (ref. 400/50)
Antioxidants in Vegetable Oils
Complimentary data

Good separation of phenolic antioxidants

Separation of tocopherols not complete, despite long run time

Good separation of all tocopherols in reasonable time

Separation of phenolic antioxidants not good, long run time

HPLC

SFC

October 12, 2015
Antioxidants in Vegetable Oils
Separation of tocopherols by SFC with UV/MS detection

*no β-tocotrienol as standard available
Antioxidants in Vegetable Oils
Analysis of tocopherols in oil samples by SFC/MS

Deep frying oil

Sunflower oil

Rapeseed oil

Tocomix
A new Flame Ionization Detector (FID) for Agilent 1260 Infinity SFC System
A new Flame Ionization Detector (FID) for Agilent 1260 Infinity SFC System

- In cooperation with SIM Scientific Instruments Manufacturer GmbH
- Addresses ASTM 5186 and other standard methods for determining unsaturation in oil, diesel and biofuels
- Added FID detector to SFC
Integrated in OpenLab Chemstation
Online Screen Status of FID and PAL
ASTM Performance – Hydrocarbon Mix

<table>
<thead>
<tr>
<th>Substance</th>
<th>Weight in [g]</th>
<th>w [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexadecane (C16)</td>
<td>5,738</td>
<td>73.9</td>
</tr>
<tr>
<td>Toluene (T)</td>
<td>1,605</td>
<td>20.7</td>
</tr>
<tr>
<td>Tetrahydronaphthalene (THN)</td>
<td>0,259</td>
<td>3.3</td>
</tr>
<tr>
<td>Naphthalene (N)</td>
<td>0,162</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Column: RX-Sil, 250x4.6 mm, 5µm

Mob. phase: CO$_2$ @ 1.5 mL/min
BPR 150 bar
→ head pressure 205 bar

FID:
- Temp: 350°C
- H$_2$: 65 mL/min
- Air: 350 mL/min
- CO$_2$: 60 mL/min*
  *gas flow @ FID

ASTM Requirements:
- Reproducibility RT: <0.5 %, n=10

Obtained values:
- C16: 0.07 %
- T: 0.22 %
- THN: 0.17 %
- N: 0.15 %
Method Development
Screening of chiral stationary phases and mobile phase modifiers

Column selection
• Up to eight short (≤ 100 mm) or four long (> 100 mm) columns
• Six long columns (> 100 mm) with 3rd TCC
• For chiral and achiral column screening
Method Development
Screening of chiral stationary phases and mobile phase modifiers

Solvent selection
• Up to three mobile phase modifiers with optional, built-in solvent selection valve
• Up to twelve mobile phase modifiers with external valve drive and 12-pos/13-port valve head
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### Application Notes (1)

<table>
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<tr>
<td>5991-3859EN</td>
<td>Fast Analysis of Chiral and Structurally Related Isomers Using Supercritical Fluid Chromatography Mass Spectrometry</td>
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<tr>
<td>5991-1546EN</td>
<td>Analysis of antioxidants in vegetable oils using the Agilent 1260 Infinity Hybrid SFC/UHPLC System with MS detection</td>
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<tr>
<td>5991-1456EN</td>
<td>Transfer of USP Cholecalciferol Normal-phase HPLC Method to SFC Using the Agilent 1260 Infinity Hybrid SFC/UHPLC System</td>
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<tr>
<td>5991-1143EN</td>
<td>Fast determination of residual glycerol and glycerides in biodiesel by SFC/MS using the Agilent 1260 Infinity Analytical SFC System</td>
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<tr>
<td>5991-0988EN</td>
<td>Coupling the Agilent 1260 Infinity Analytical SFC System to an Agilent 1260 Infinity Evaporative Light Scattering Detector</td>
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<tr>
<td>5991-0987EN</td>
<td>Analysis of triglycerides in vegetable oils using the Agilent 1260 Infinity Analytical SFC System with evaporative light scattering detection</td>
</tr>
<tr>
<td>5991-0986EN</td>
<td>Fast screening of impurities in biodiesel using the Agilent 1260 Infinity Analytical SFC System in combination with evaporative light scattering detection</td>
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<tr>
<td>5991-0514EN</td>
<td>Injecting variable volumes using the partial loop-fill method with the Agilent 1260 Infinity Analytical SFC System</td>
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<tr>
<td>5991-0395EN</td>
<td>Agilent Application Solution: Transfer of a USP method for tolazamide from normal phase HPLC to SFC using the Agilent 1260 Infinity Hybrid SFC/UHPLC System Improving peak shape and providing wider UV selectivity</td>
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<tr>
<td>5991-0277EN</td>
<td>Agilent Application Solution: Transfer of a USP method for prednisolone from normal phase HPLC to SFC using the Agilent 1260 Infinity Hybrid SFC/UHPLC System Saving time and costs</td>
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<tr>
<td>5990-9598EN</td>
<td>Determination of polymer additives and migration products prevalent in food packaging material Using the Agilent 1260 Infinity SFC System with the Agilent 6130 Single Quadrupole LC/MS System</td>
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### For more Information

#### Application Notes (2)

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<td>Determination of phthalate migration from toys Using the Agilent 1260 Infinity Analytical SFC System with an Agilent 6130 Single Quadrupole LC/MS</td>
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<td>5990-9594EN</td>
<td>Enantionmer separation of acidic compounds Using Daicel CHIRALPAK QN-AX and QD-AX columns and the Agilent 1260 Infinity Analytical SFC System</td>
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<td>5990-9514EN</td>
<td>Agilent 1260 Infinity Hybrid SFC/UHPLC System</td>
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<tr>
<td>5990-9459EN</td>
<td>Enantionmer separation of nonsteroidal anti-inflammatory drugs - Using Daicel immobilized polysaccharide-derived chiral columns and the Agilent 1260 Infinity Analytical SFC System</td>
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<td>5990-9315EN</td>
<td>Separation of enantiomers and conformers of Tofisopamon - Using Daicel immobilized polysaccharide-derived chiral columns using the Agilent 1260 Infinity Analytical SFC System</td>
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<td>5990-9195EN</td>
<td>Comparison of UV detection limits between the Agilent 1260 Infinity Analytical SFC System and an Agilent 1200 Series LC System</td>
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<td>5990-7972EN</td>
<td>Agilent 1260 Infinity SFC/MS Solution Superior sensitivity by seamlessly interfacing to the Agilent 6100 Series LC/MS system</td>
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<td>5990-7934EN</td>
<td>Performance optimization using the Agilent 1260 Infinity Analytical SFC system</td>
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<td>5990-6934EN</td>
<td>High resolution separations by supercritical fluid chromatography using a coupled column approach with the Agilent 1260 Infinity Analytical SFC System</td>
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<tr>
<td>5990-6413EN</td>
<td>Sensitive determination of impurities in achiral pharmaceuticals by supercritical fluid chromatography using the Agilent 1260 Infinity Analytical SFC System</td>
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<tr>
<td>5990-6412EN</td>
<td>Fast and ultra-fast SFC analysis using the Agilent Analytical SFC System with the Aurora SFC-Fusion A5 - Effect of particle size and column length on speed</td>
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<tr>
<td>5990-5969EN</td>
<td>Chiral impurity analysis and enantiomeric excess determination with the Agilent 1260 Analytical SFC system</td>
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For more Information


• Cost calculator - calculates operating costs of SFC vs. LC

• Hybrid SFC/UHPLC Video

• Hybrid SFC/UHPLC E-seminar
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Summary

Unique Selectivity / Orthogonal to LC
• Replacement for Normal Phase Chromatography
• Complementary to RP-LC or HILIC

CO₂ Mobile Phase / Very Little Organics
• High analysis speed – faster than LC!
• Lower operational costs and a green technology!!!

Broad Detector Compatibility
• UV, MS, ELSD...

Chirals and more!
• Premier choice for chiral compounds
• Many applications for achiral compounds!
• Polar and non-polar compounds in a single run

Wide application range
• LC-like robustness & sensitivity combined with SFC speed and orthogonal separation selectivity