

Development of an orthogonal SFC method for mometasone furoate impurity analysis

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What role is SFC playing in Pharm industry

- Preferred tool for **chiral** separation in discovery & process chemistry
- Competitive tool for **achiral** separation in discovery & process chemistry
- Some usage in API **chiral** separation in process chemistry (GMP)
- Less popular in API **achiral** separation in process chemistry (GMP)
- Few usage in drug product analysis



SFC can do more:

- Chiral and achiral
- Non-GMP and GMP
- Drug substance and drug product

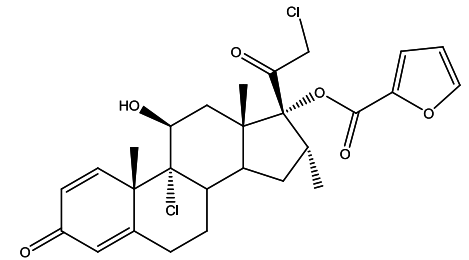
Develop an SFC method for MF impurity analysis

Goals:

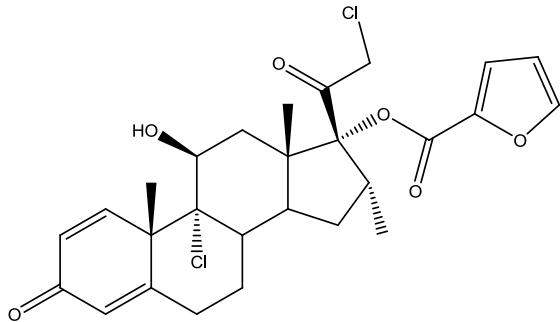
- **Use MF as a case study to evaluate SFC for achiral impurity profiling**
- **Showcase the potential of SFC in achiral analysis in regulated area -- Not just faster!**
- **Identify areas of improvement**

Mometasone furoate franchise

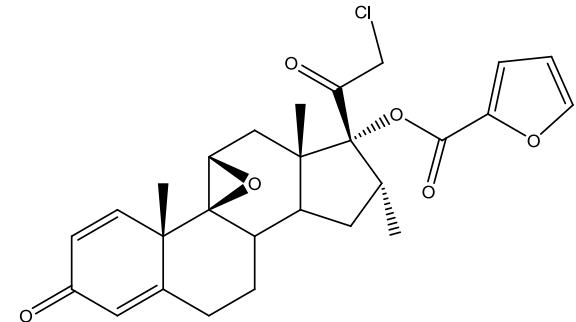
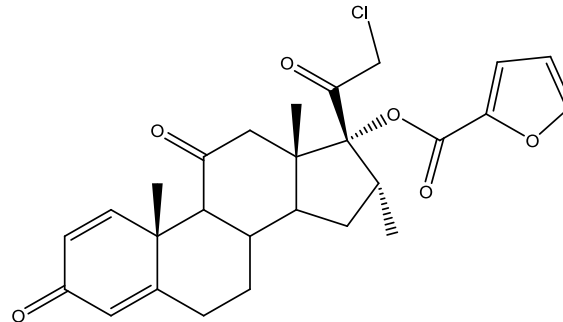
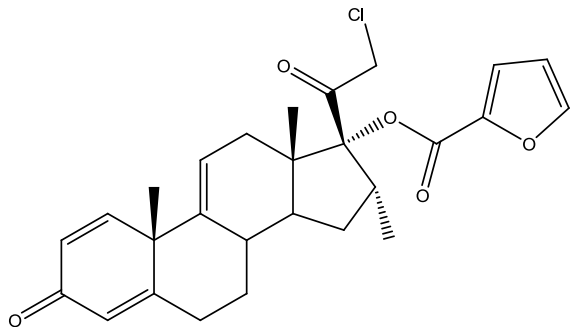
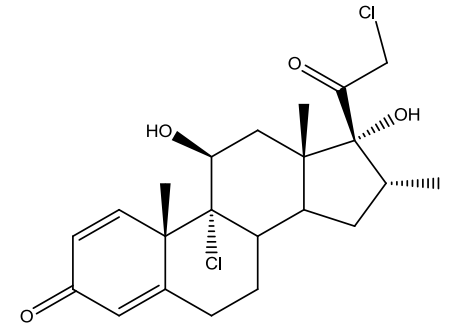
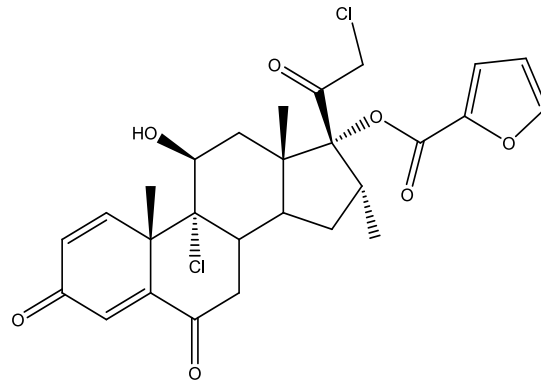
- A highly potent glucocorticoid anti-inflammatory agent
- The active ingredient of several drug products



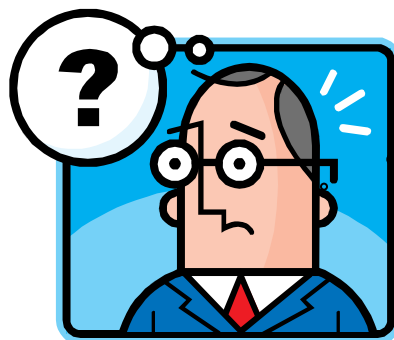
Mometasone furoate and its major impurities



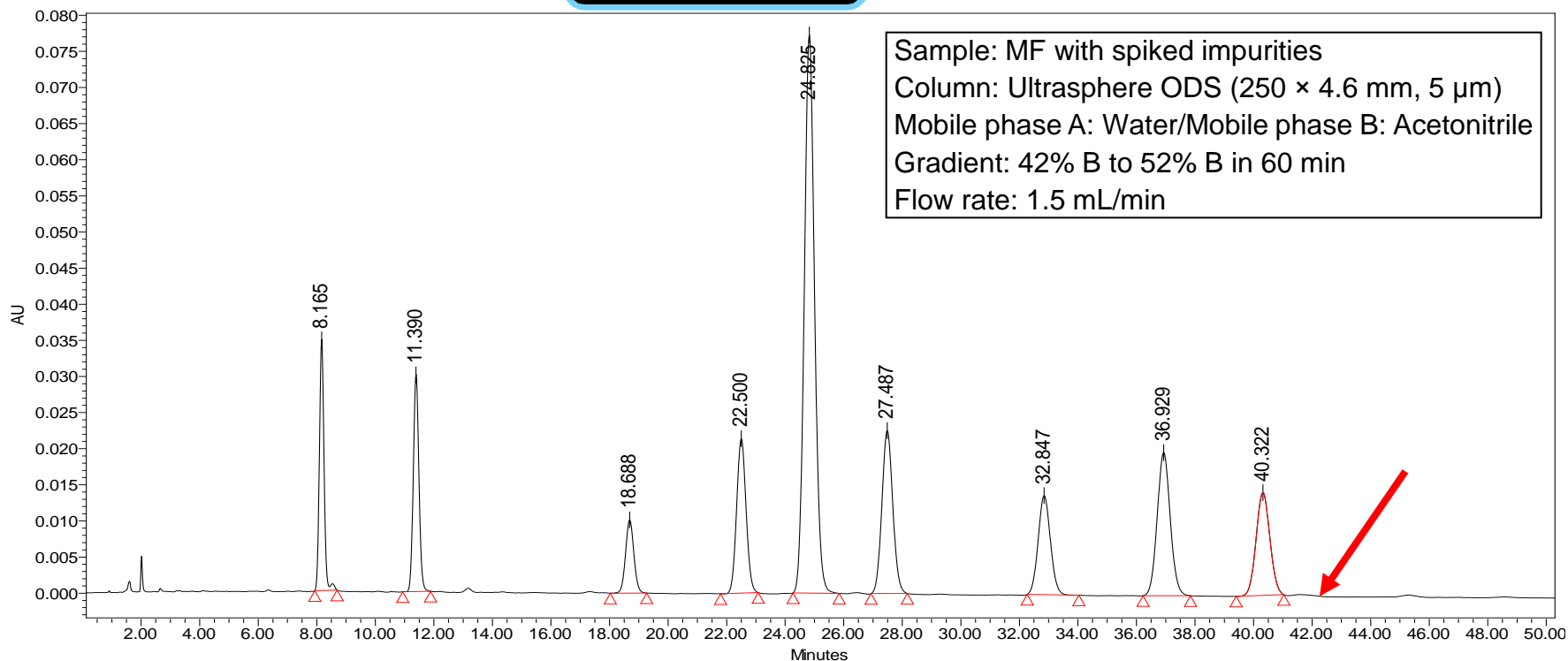
Mometasone furoate



Current RPLC method for MF impurity profiling*



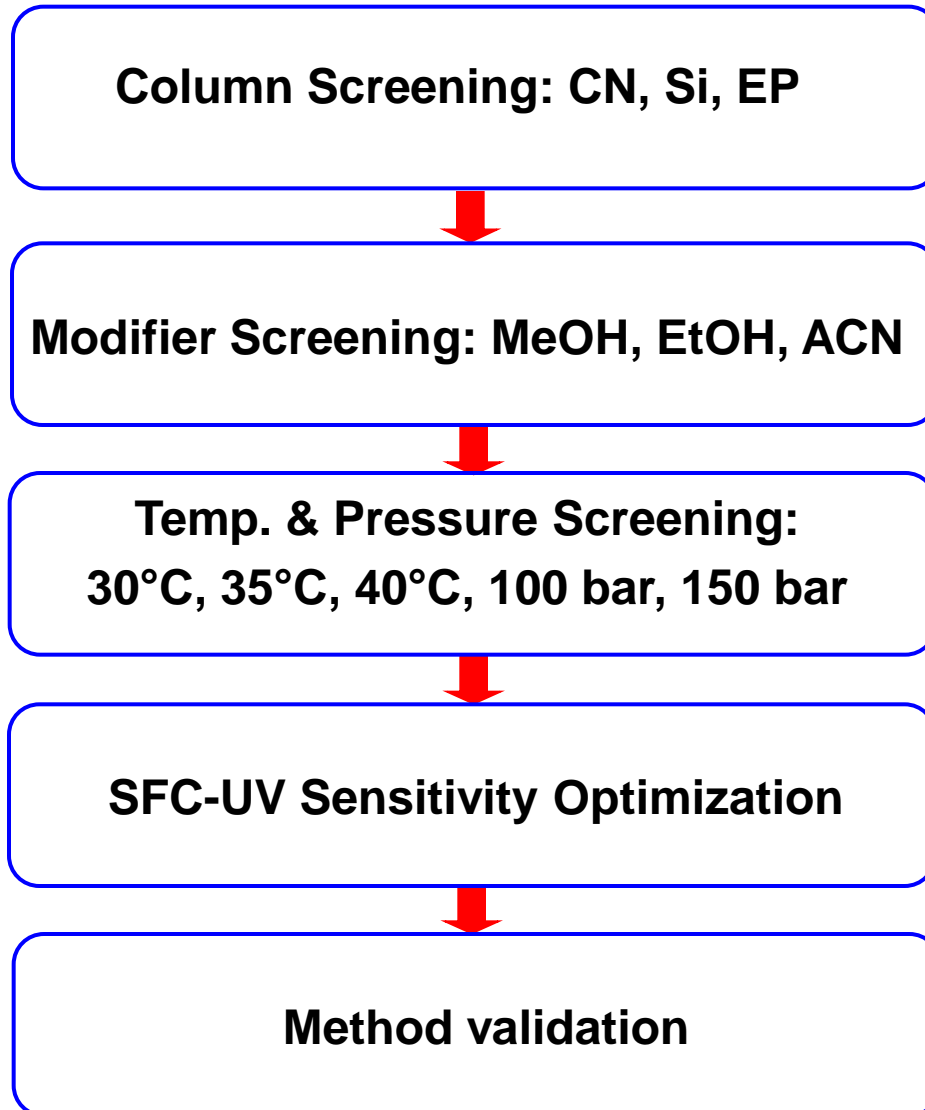
What if I use SFC



Part A:

SFC method development and optimization

Experiment flow – stepwise approach



SFC & HPLC Instrument and software

All SFC experiments were performed on:

TharSFC Method Station Analytical System

- Solvent selector**
- Column selector**
- Waters 2998 PDA detector**

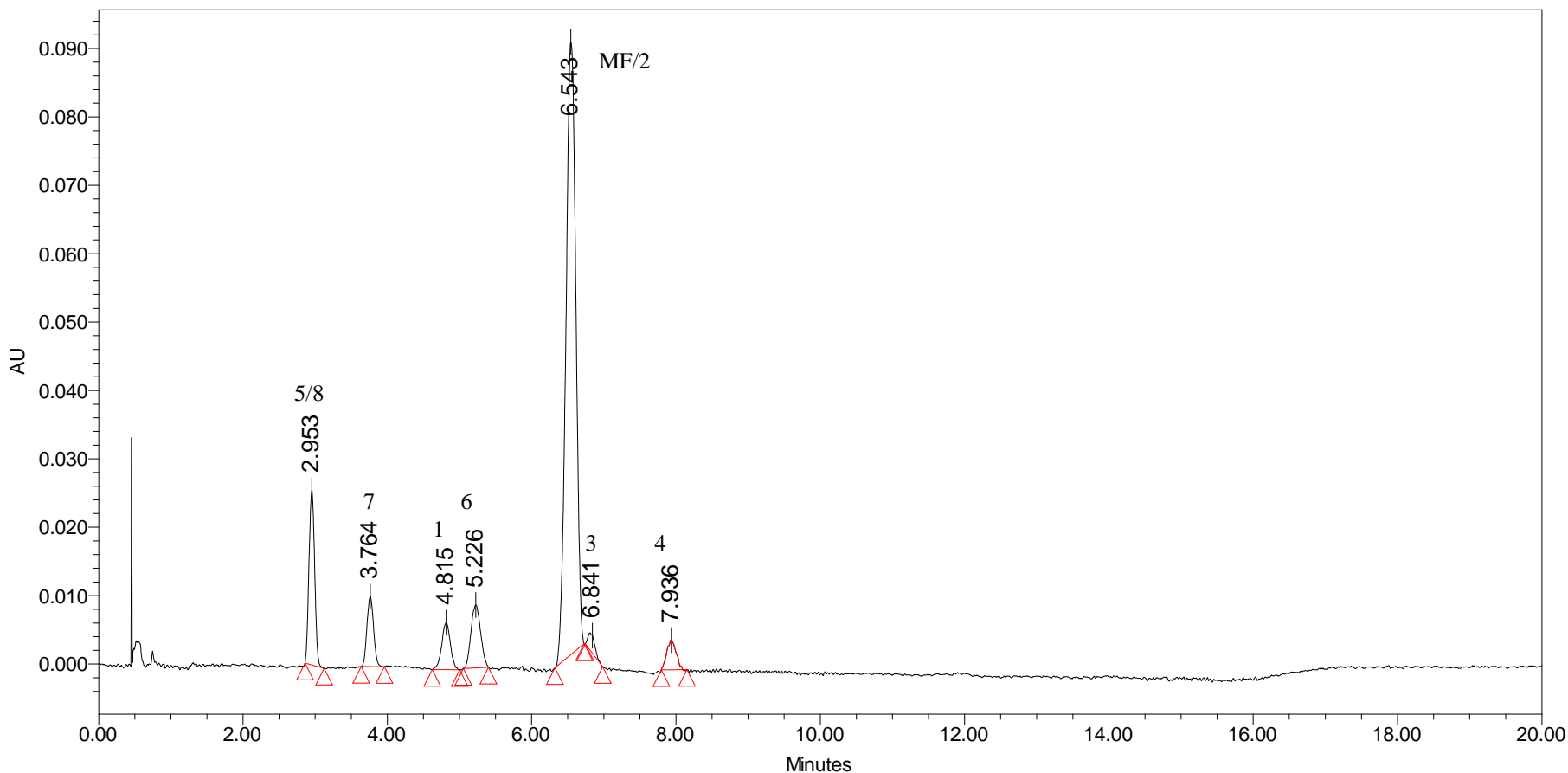
Instrument control and data collection: Empower 2

All HPLC experiments were performed on:

Alliance 2690 HPLC System equipped with 2996 PDA detector

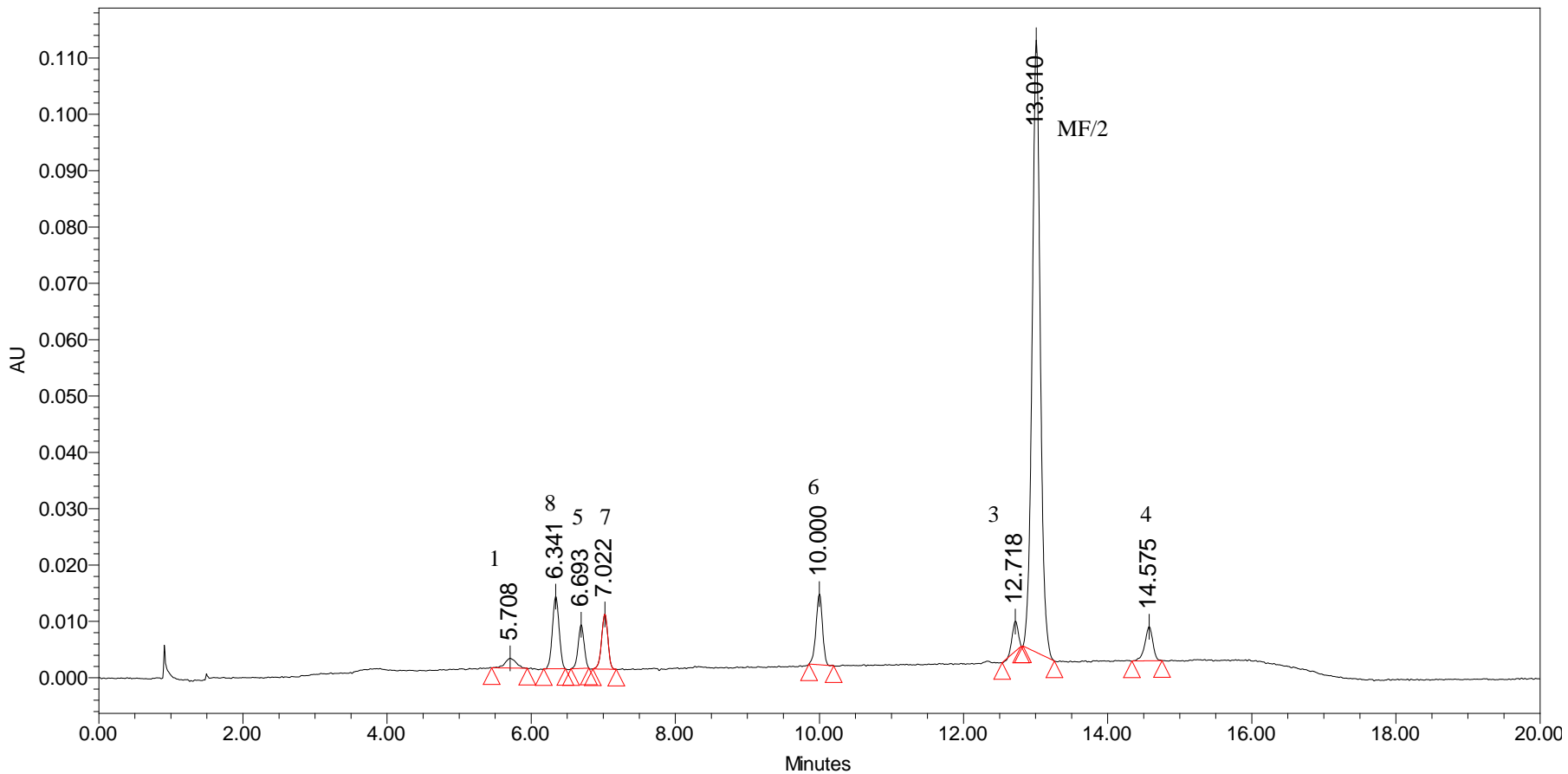
Instrument control and data collection: Empower 2

SFC Column Screening – Cyano column



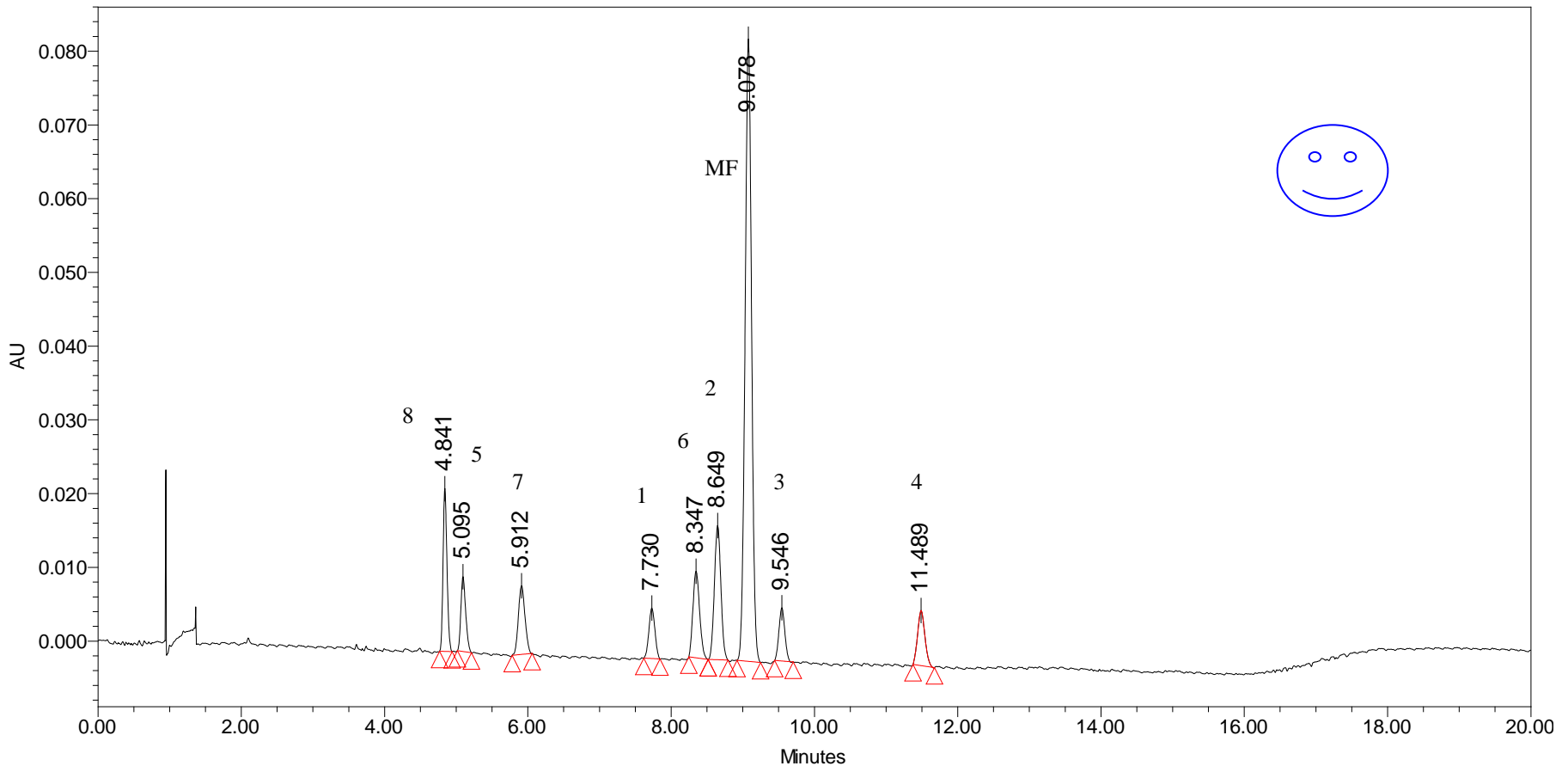
Cyano: 250 × 4.6 mm, 5 μm, Restek
Mobile phase: 4 mL/min, CO₂ 100 bar, 30 °C
Gradient: 5% MeOH to 20% MeOH in 15 min

SFC Column Screening – 2-Ethylpyridine column



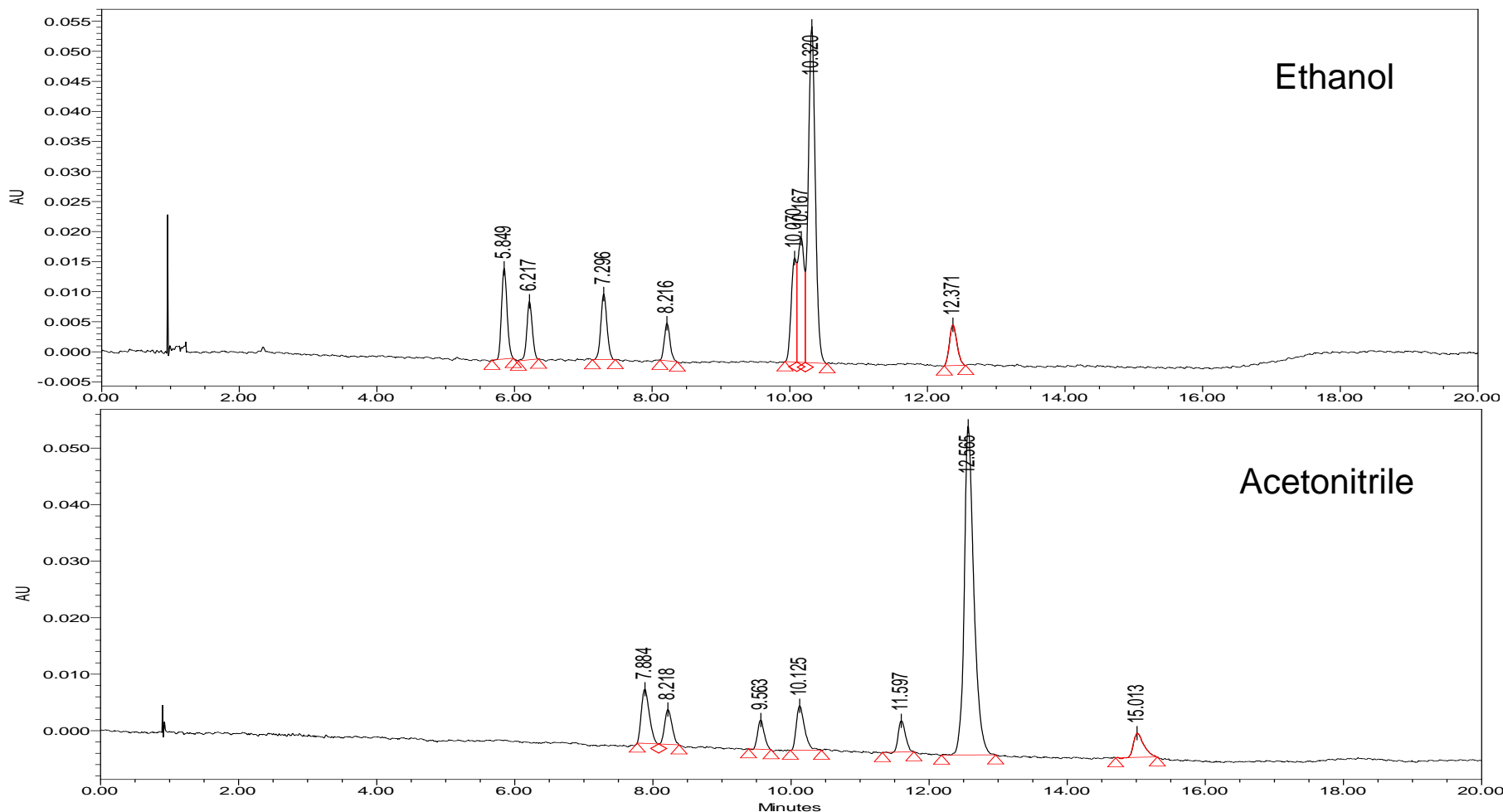
2-EP: 250 × 4.6 mm, 5 μm, Princeton Chromatography
Mobile phase: 4 mL/min, CO₂ 100 bar, 30 °C
Gradient: 5% MeOH to 20% MeOH in 15 min

SFC Column Screening – Silica column



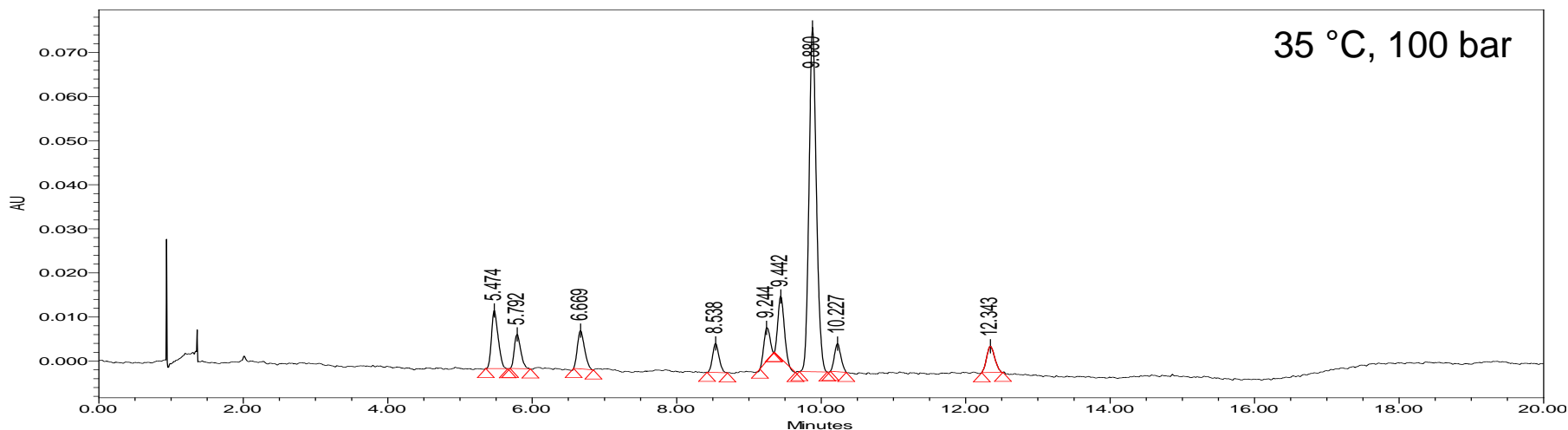
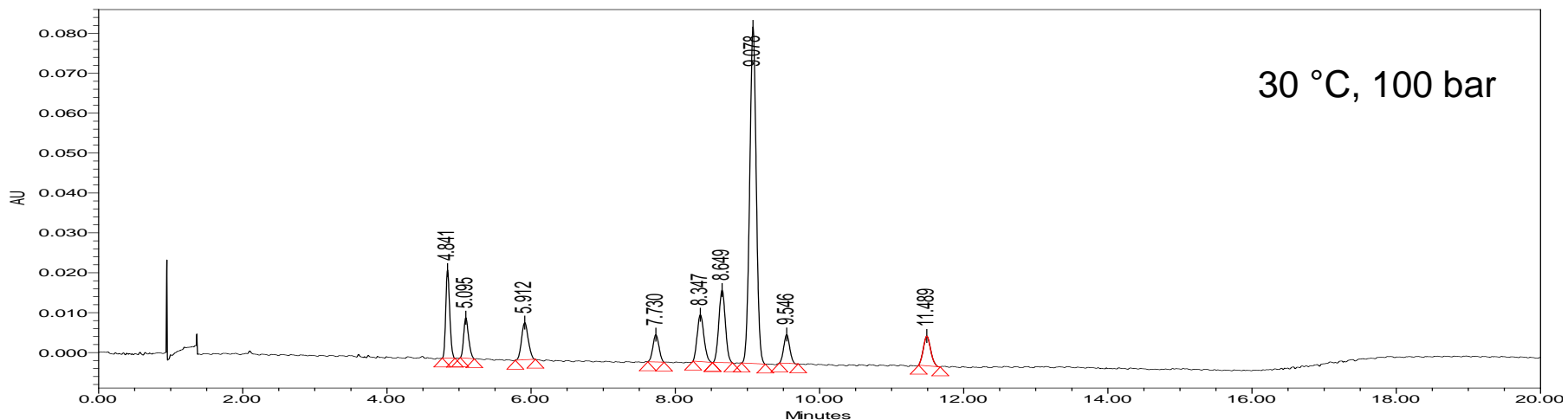
Silica: 250 × 4.6 mm, 5 μm, Kromasil
Mobile phase: 4 mL/min, CO₂ 100 bar, 30 °C
Gradient: 5% MeOH to 20% MeOH in 15 min

SFC Modifier Screening



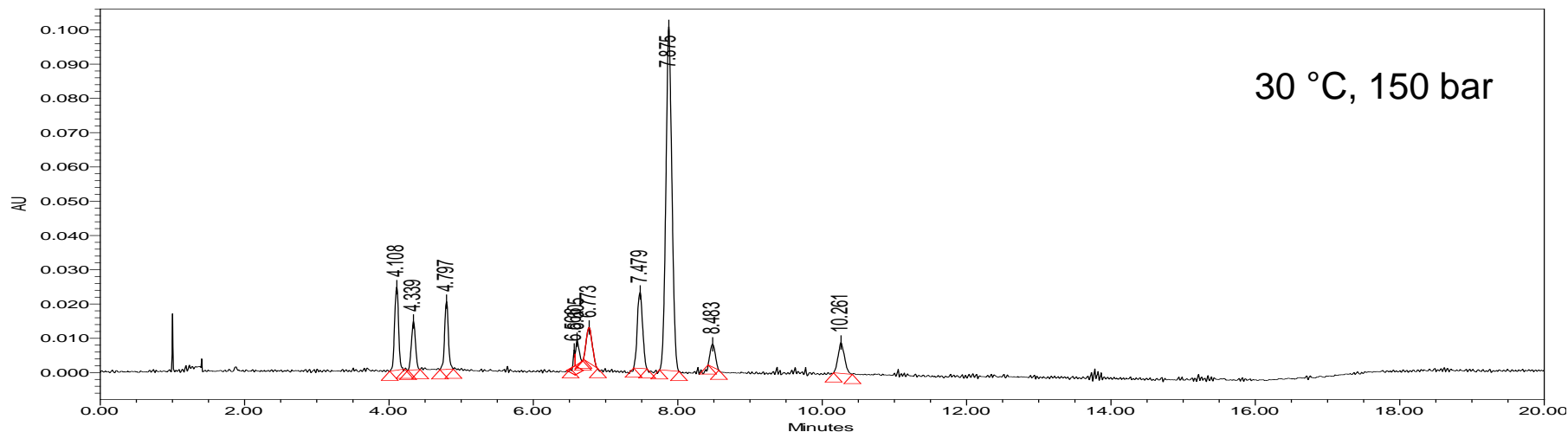
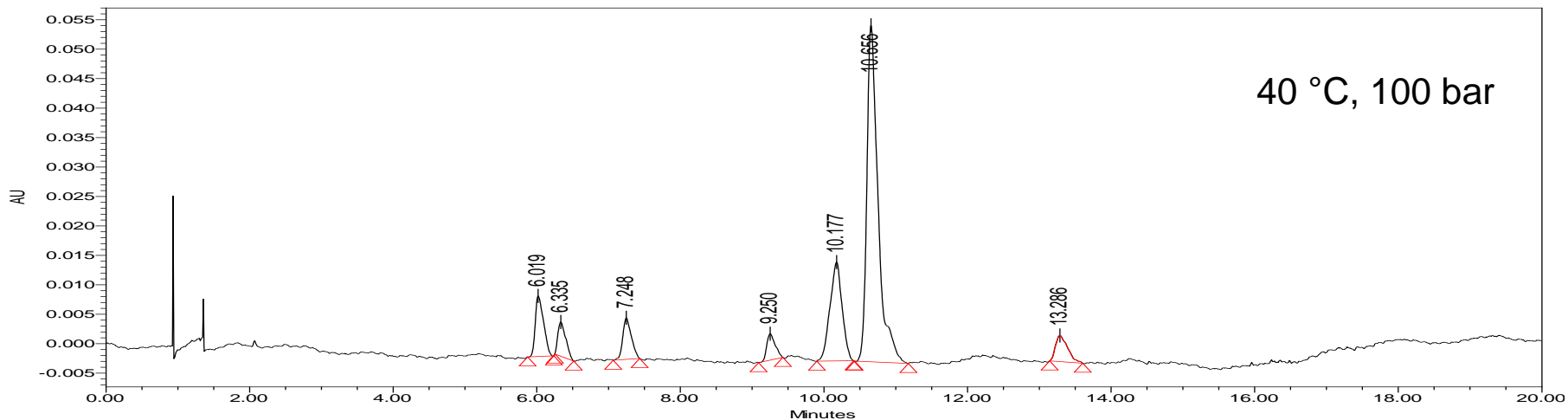
Silica: 250 × 4.6 mm, 5 μm, Kromasil
Mobile phase: 4 mL/min, CO₂ 100 bar, 30 °C
Gradient: 5% modifier to 20% modifier in 15 min

Impact of temperature and pressure



Silica: 250 × 4.6 mm, 5 μm, Kromasil
Mobile phase: 4 mL/min, CO₂
Gradient: 5% MeOH to 20% MeOH in 15 min

Impact of temperature and pressure (Cont'd)



Silica: 250 × 4.6 mm, 5 μm, Kromasil

Mobile phase: 4 mL/min, CO₂

Gradient: 5% MeOH to 20% MeOH in 15 min

Part B:

Improve SFC-UV sensitivity

Improve SFC-UV sensitivity

Sensitivity is the key for drug impurity analysis:

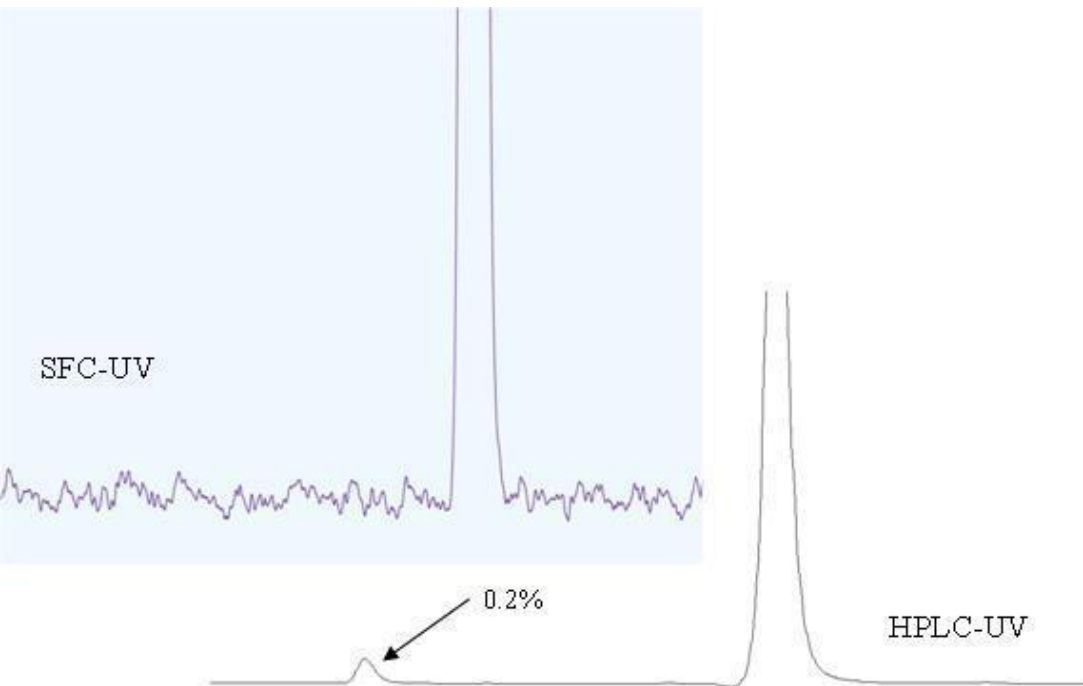
- ❖ Drug safety and quality control
- ❖ Reporting threshold: 0.05% impurity in Drug Substance (≤ 2 g/day), ICH Q3A
- ❖ Reporting threshold: 0.1% degradation product in Drug Product (≤ 1 g/day), ICH Q3B



Why SFC-UV is less sensitive (vs. HPLC-UV)

Three main sources of noise:

- ❖ Electronic: noise from detector system
- ❖ Mechanical: BPR, pump
- ❖ Thermal: endothermic process during depressurization



Anton, K.et.al. *Analusis*, 1999, 27, 691
Helmy, R. et.al. *Chirality*, 2007, 19, 787
Wang, Z, et.al, *Am. Pharm. Rev*, 2009, 5, 59

How to get better sensitivity on SFC-UV

- Software filtering: reduce non-wavelength dependent noise ^{1,2}
- Hardware modification: reduce mechanical and thermal noise ³
- Next generation SFC



- 1 Chen, R., LC-GC Application Notebook, 2009, Sep
2. Wang, Z, et.al, *J. Chromatogr. A*, 2011, 1218, 2311
3. Helmy, R. et.al. *Chirality*, 2007, 19, 787

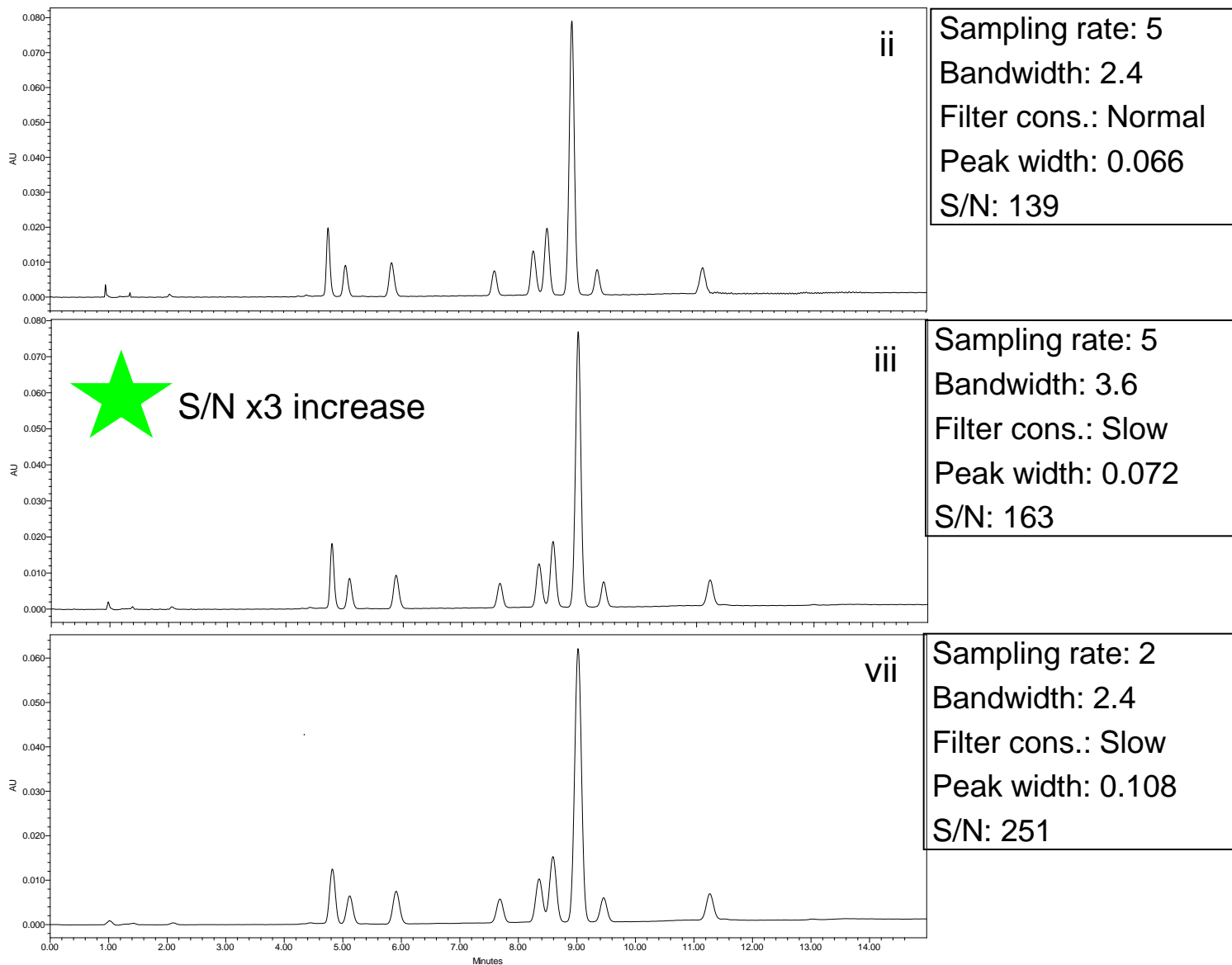
Reference Wavelength Compensation to increase S/N

PDA detector settings				Without wavelength compensation		With wavelength compensation		Improvement in sensitivity
	Sampling rate	Bandwidth	Filter constant	Peak width	S/N ^a	Peak width	S/N ^b	(S/N ^b)/(S/N ^a)
i	5	2.4	Slow	0.072	67	0.072	151	2.2
ii*	5	2.4	Normal	0.066	44	0.066	139	3.2
iii*	5	3.6	Slow	0.073	57	0.072	163	2.9
iv	5	3.6	Normal	0.066	62	0.066	122	2
v	5	4.8	Slow	0.072	62	0.072	142	2.3
vi	5	4.8	Normal	0.066	54	0.067	115	2.1
vii*	2	2.4	Slow	0.108	62	0.108	251	4
viii	2	2.4	Normal	0.077	71	0.077	170	2.4
ix	2	3.6	Slow	0.108	52	0.108	190	3.7
x	2	3.6	Normal	0.077	47	0.077	159	3.4
xi	2	4.8	Slow	0.109	63	0.108	175	2.8
xii	2	4.8	Normal	0.077	43	0.077	162	3.8

Detection wavelength: 245 nm

Compensation wavelength: 400-450 nm

Ref. Wavelength Compensation to increase S/N (Cont'd)



Part C:

Comparison of SFC method with RPLC method

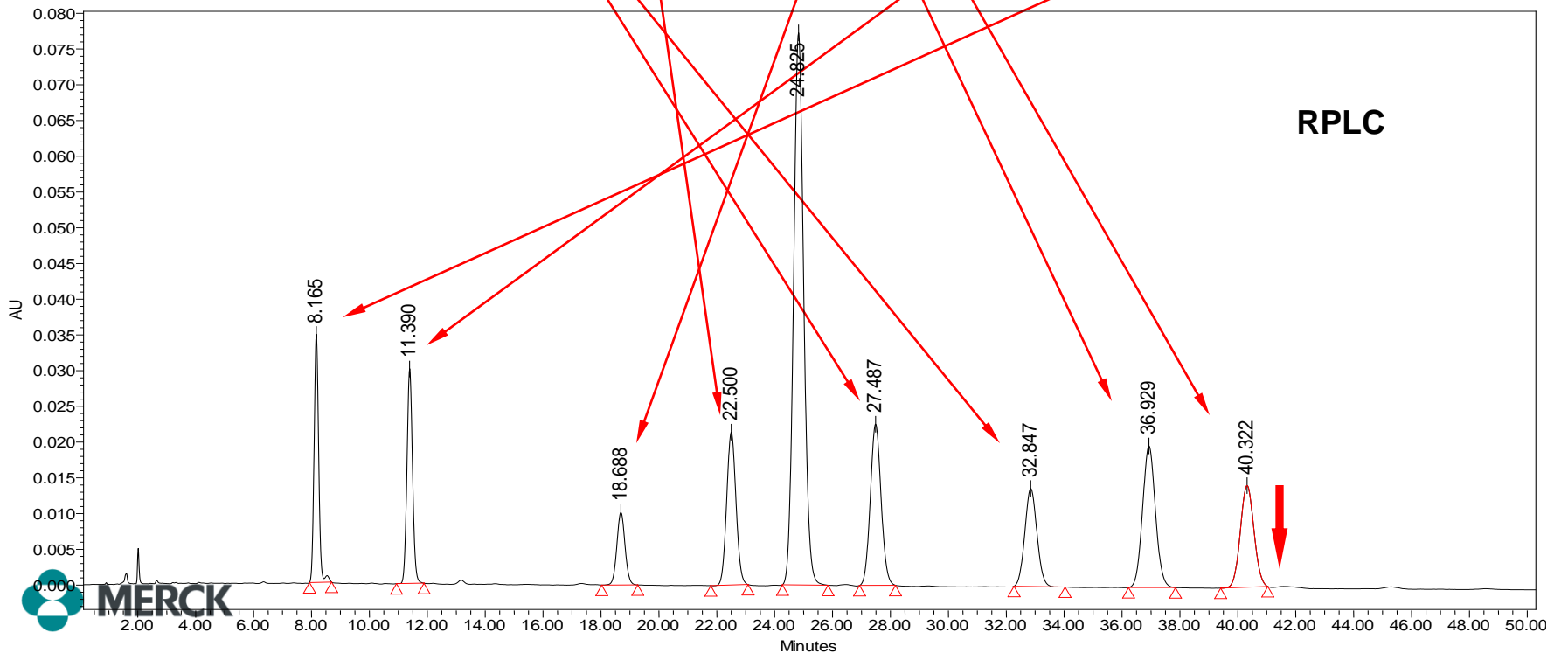
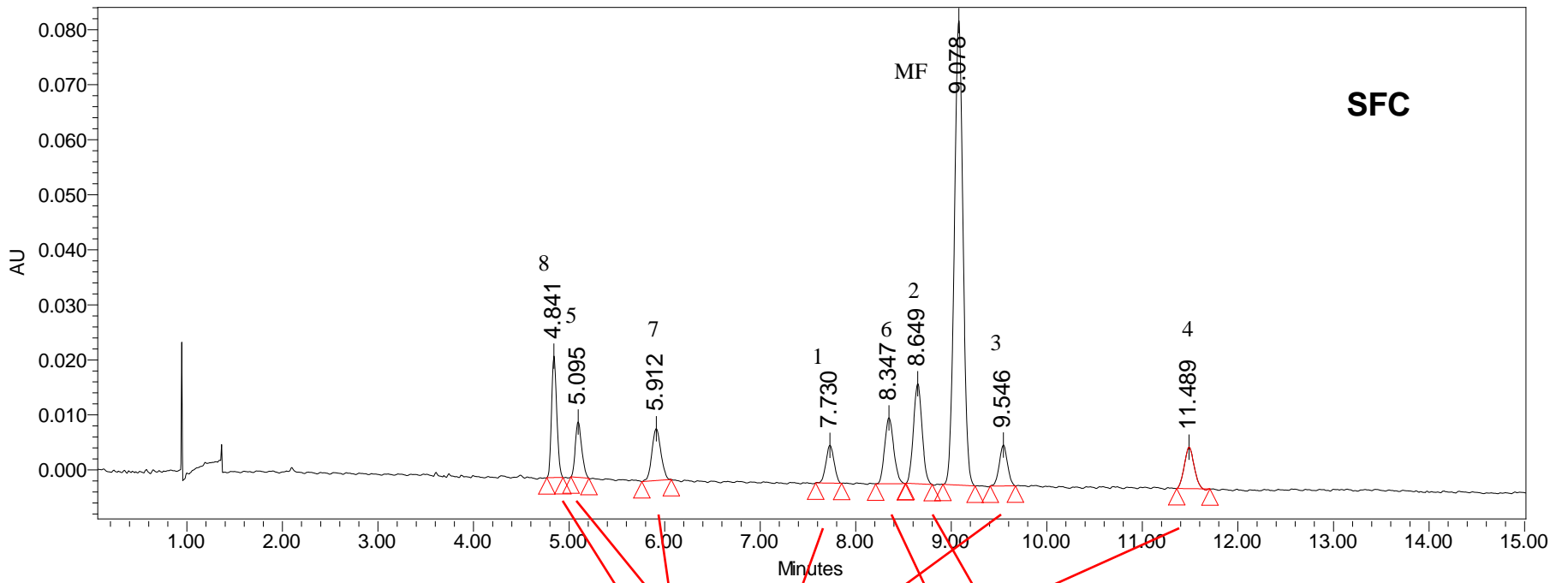
Comparison of RPLC and SFC method validation results

		RP-HPLC method	SFC method
Sample concentration		0.2 mg/mL	2.0 mg/mL
Linearity		0.9999	0.9999
Accuracy	Assay level ^a	99.1% - 100.7%	99.8% - 101.6%
	Impurity level ^b	96.6% - 115.4% ^c	88.3% - 104.7% ^c
Precision	Assay level ^a	0.4%	0.7%
	Impurity level ^b	1.9% - 5.0%	1.4% - 5.4%
Limit of Quantitation		0.05% (or 0.1 µg/mL)	0.05% (or 1.0 µg/mL)

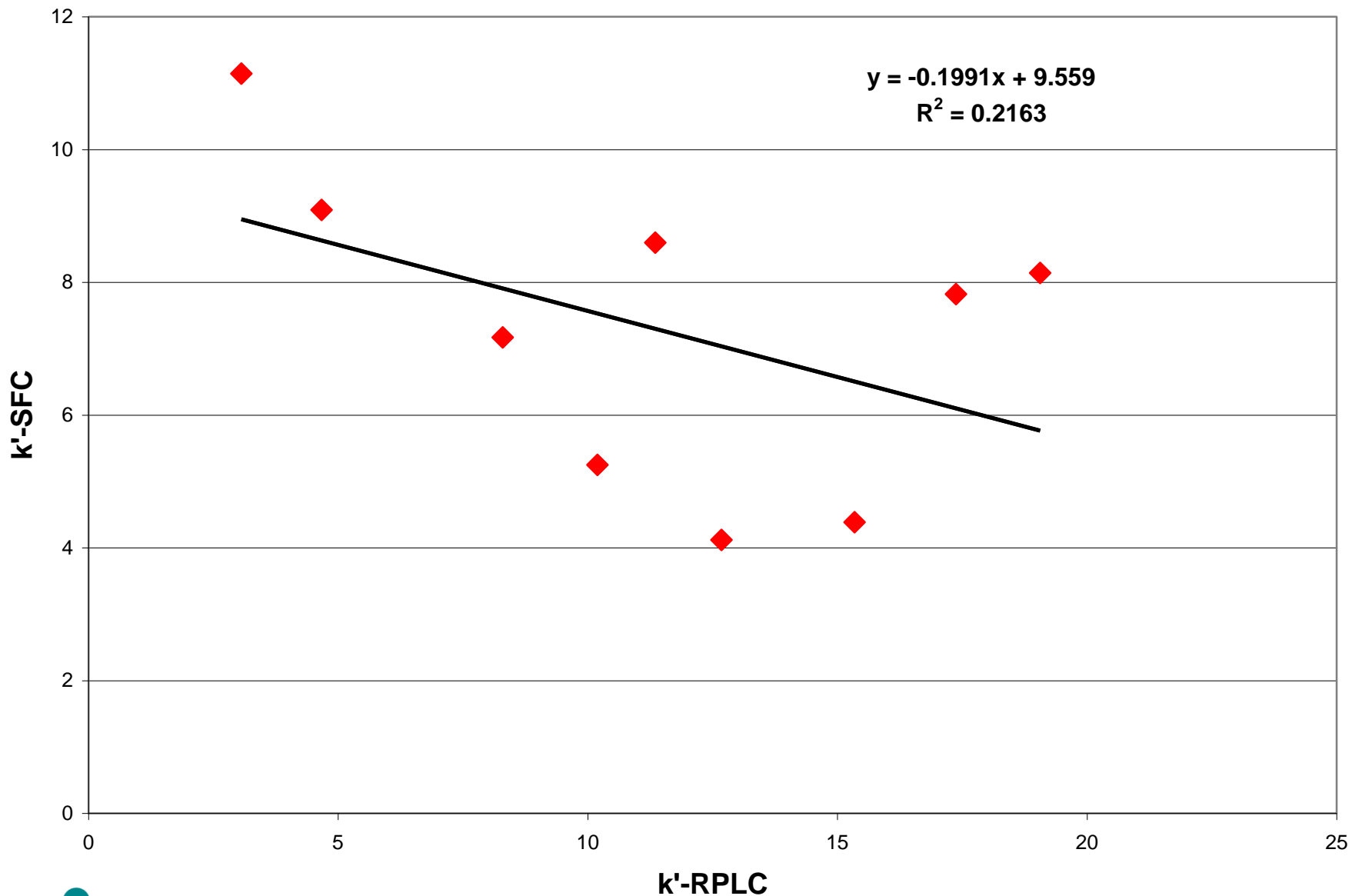
a. Six preparations (n = 6)

b. Six preparations of spiked individual impurities

c. The average recovery of each impurity was reported.



Orthogonal selectivity of SFC method vs. RPLC method



Summary

- The study demonstrated the potential and uniqueness of SFC in achiral impurity analysis:
 - Faster, cheaper, greener, and complementary to RPLC
- SFC's orthogonal selectivity should offer great value in pharmaceutical impurity profiling
- The SFC method is comparable to the current mometasone furoate impurity HPLC method for most chromatographic criteria
- With improved sensitivity, SFC could become an active player of chiral and achiral analysis in regulated environment.

The Outlook

The scoreboard of SFC application in pharmaceutical R&D (0-5)

	Research	Development			
	Drug Substance	Drug Substance		Drug Product	
	Non-GMP	Non-GMP	GMP	Non-GMP	GMP
Chiral	5	5	2	1	0
Achiral	3	3	0	0	0

To get higher score in these areas we need:

- ❖ Qualified SFC instrument with improved sensitivity
- ❖ Unique SFC applications to solve challenging tasks
- ❖ Sample preparation techniques for drug product

Acknowledgement

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