ISPSO
INSTITUT DES SCIENCES
PHARMACEUTIQUES
DE SUISSE OCCIDENTALE

UNIVERSITÉ
DE GENÈVE

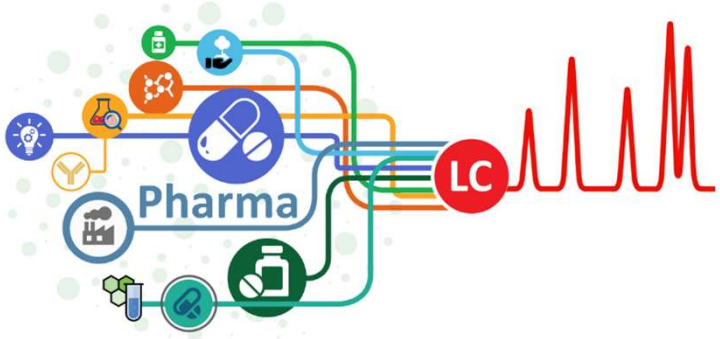
L'analyse pharmaceutique est-elle en train de vivre une révolution?

Davy GUILLARME

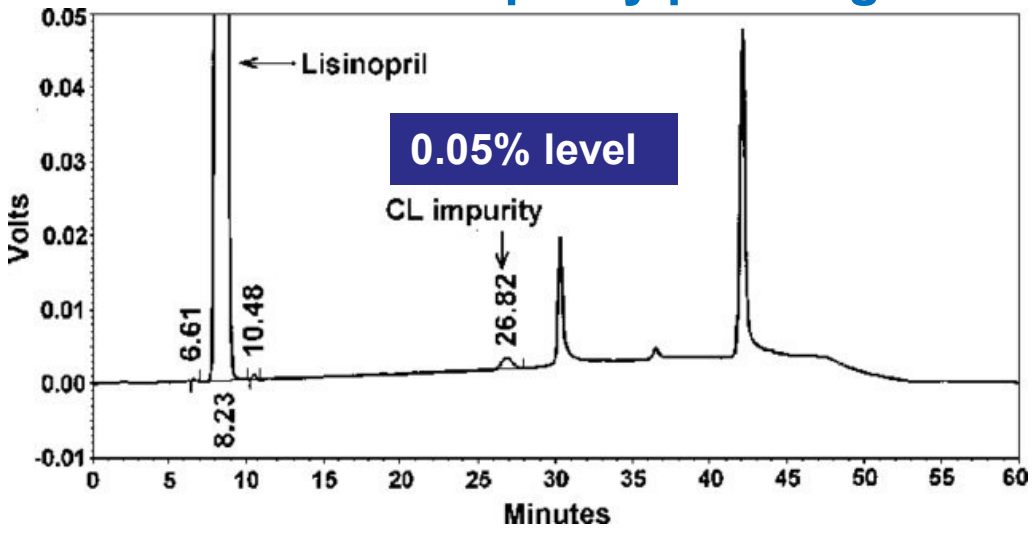
Remerciements: *Prof. Jean-luc Veuthey, Prof. Serge Rudaz et autres collègues de notre laboratoire*

Analysis in the pharmaceutical industry

- Pharmaceutical industry is one of the most regulated production worldwide, since the drug products have to be **safe and effective.**
- The levels of synthesis impurities and degradation products in active pharmaceutical ingredients (API) have to be **strictly controlled** and should meet the specifications required by international authorities.
- **Chromatography** has been the technique of choice for many years to assess chemical purity of drug substances and products, and is widely used in the pharmaceutical industry from research and development to quality control (QC) laboratories.



LC-UV impurity profiling



V. Shinde, J. Pharm. Biomed. Anal. 2007, 43, 381-386

1. The quest for performance (speed / resolution)

UHPLC: use of columns packed with sub-2 µm particles + systems with extended upper pressure limit (1000-1500 bar)

High Throughput

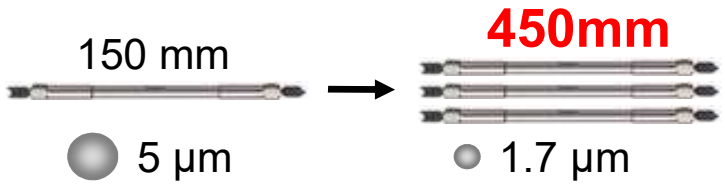
N ≈ 10'000 plates
t_{ana} < 2 min (k=10)

N ≈ cst

T_r ÷ 9

High Resolution

N ≈ 90'000 plates
t_{ana} < 20 min (k=10)



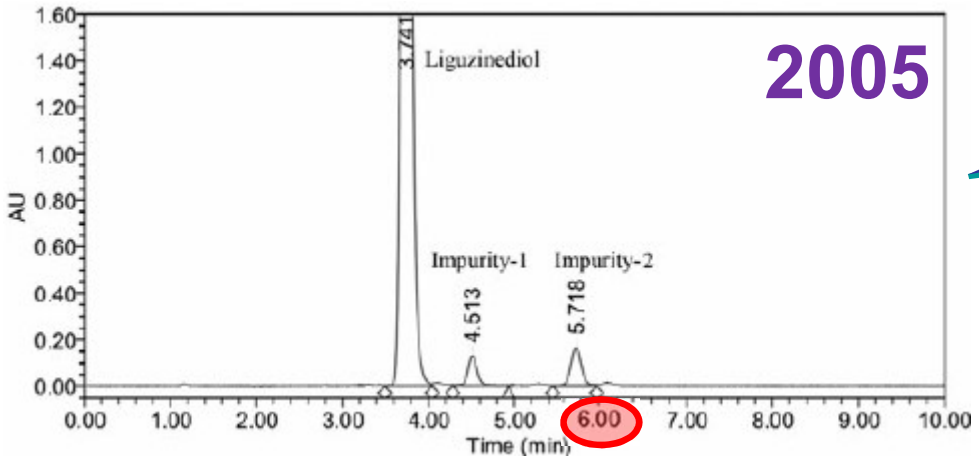
N × 9

T_r ≈ cst

S. Fekete et al., Trends Anal. Chem. 63 (2014) 2-13

Fast, very fast and ultra-fast LC analysis…

Fast analysis

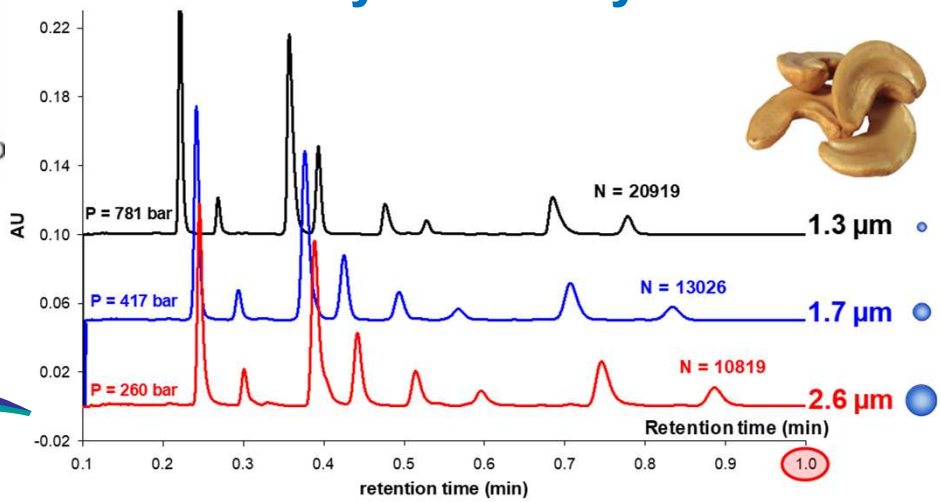


2005

D. Cheng, J. Chromatogr. Sci., 53 (2015) 1280

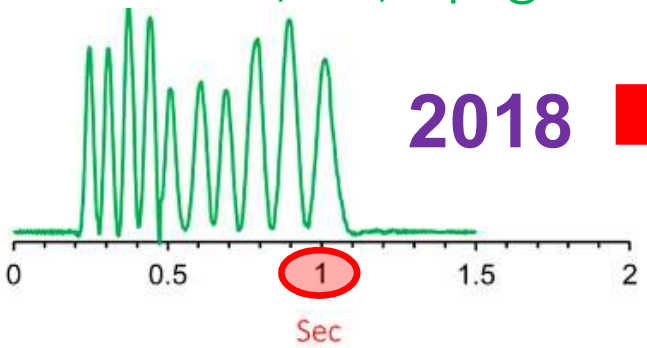
Very fast analysis

2012



Ultra fast analysis

Colonnes de 1 cm, 3 mm, 1.9 µm @ 8 mL/min



2018

D.W. Armstrong et al., Anal. Chem. 90 (2018) 3349

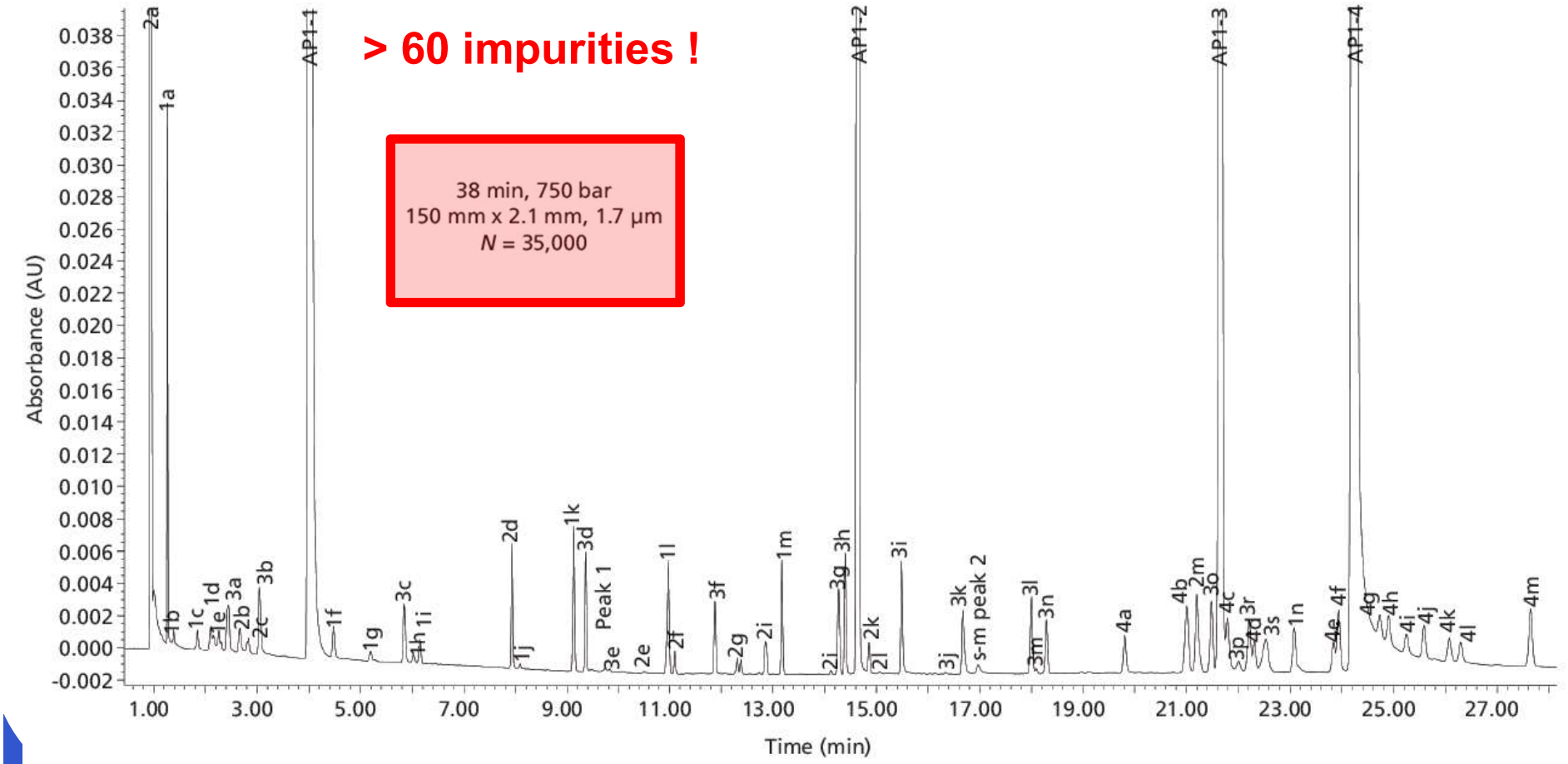
FUTURE TRENDS:

- Spectroscopy (RAMAN, near IR…)
- Sensors
- …

> 2020

High resolution LC-UV analysis

Analysis of a solution containing the four APIs present in stribild oral tablet (elvitegravir, emtricitabine, cobicistat and tenofovir) for VIH treatment, with their spiked respective impurities.



M.W. Dong et al., LC-GC North America, 2015, 28, 88-97

High resolution LC-MS analysis

Analysis of a standardized extract of *Ginkgo Biloba*

> 200 resolved peaks

Column: **300x2.1mm, 1.7µm**

T=30C, F= 200 µL/min

Gradient length: **120 minutes**

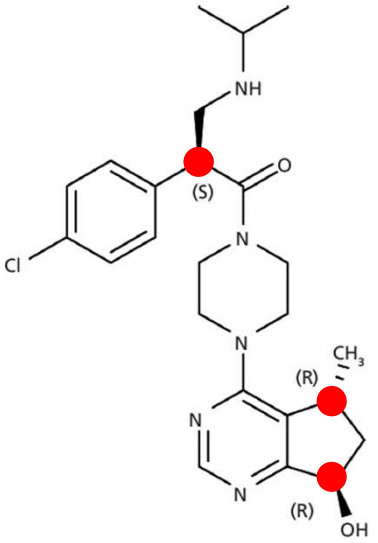
ΔP= 950 bar

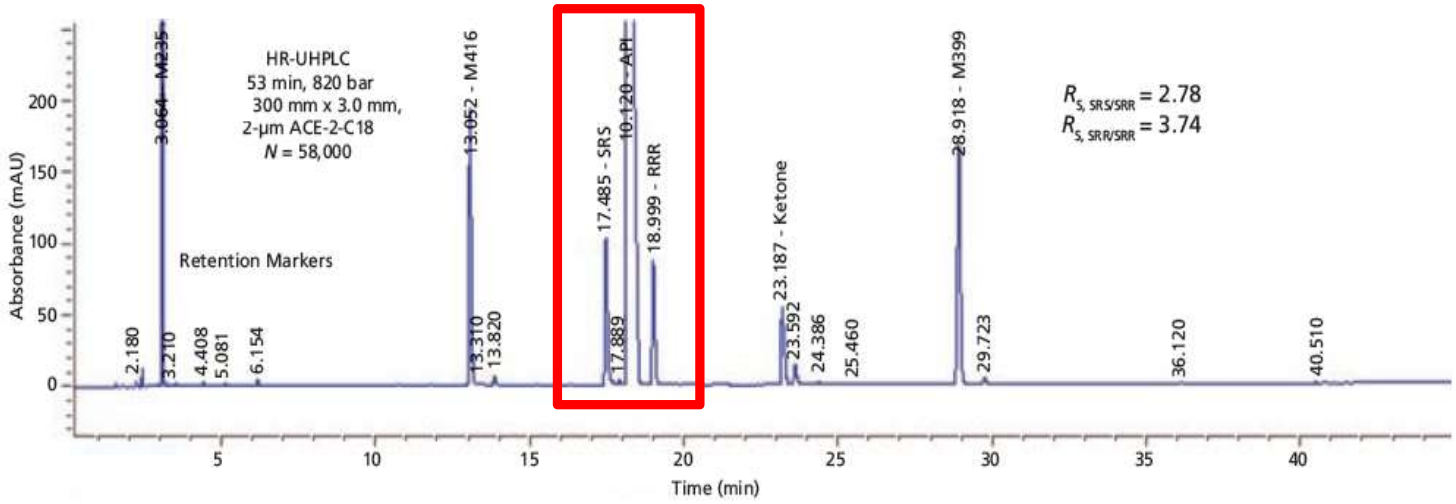
FUTURE TRENDS: 2D-LC

E. Grata et al., J. Chromatogr. A, 1216 (2009) 5660–5668

2. The quest for selectivity

Pharmaceutical products are more and more complex today (i.e. API with 3 chiral centers)





Modeling software

Tuning selectivity

SFC

Alternative selectivity

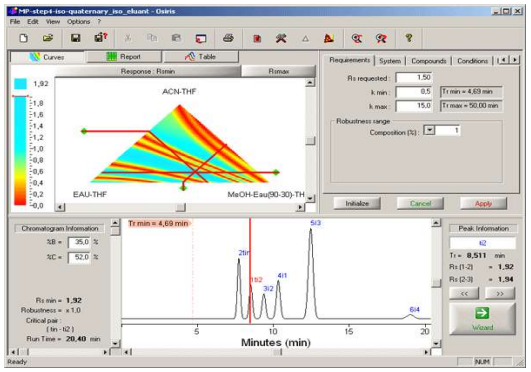
2D-LC

Improve selectivity

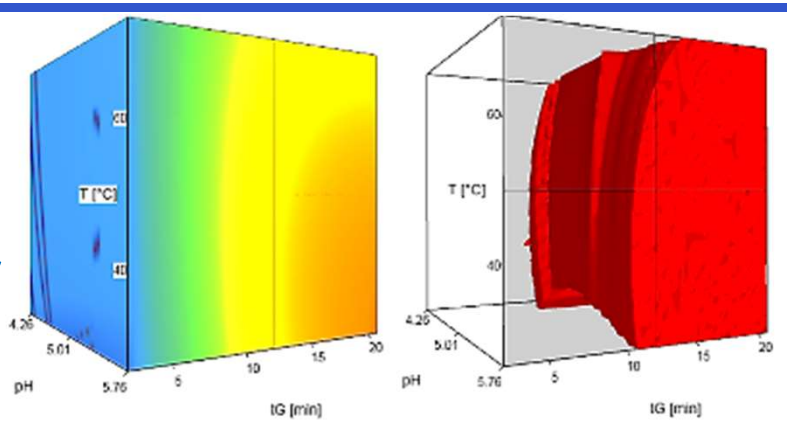
MS
IM-MS

Selective detector

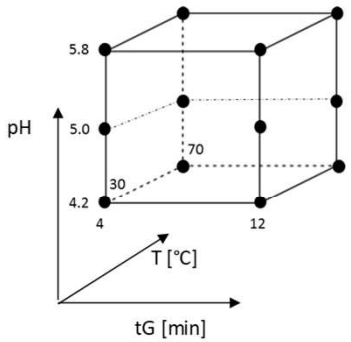
LC optimization software



- Drylab
- Chromsword
- ACD/LC simulator
- Osiris



OPTIMIZATION
(12 experiments + modeling)

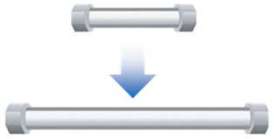


- Elution window streching
- Multilinear gradient

ROBUSTNESS
(calculation based on modeling, simulation of 729 – 17496 experiments)

Simulated full factorial design
- Gradient program
- pH
- Temperature
- Flow-rate

REFINEMENT
(calculation based on modeling + 1 verification experiment)

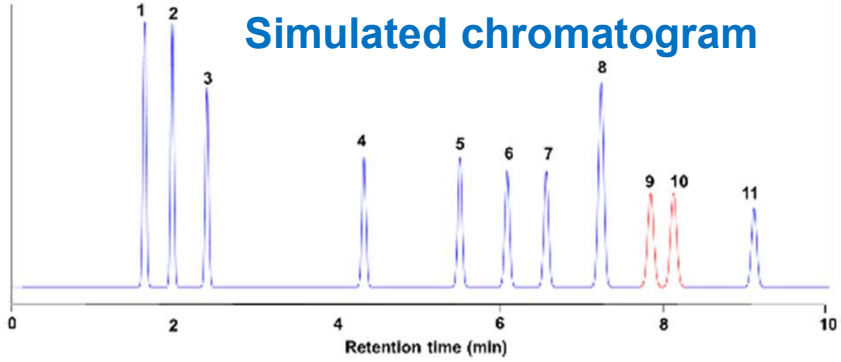


- Adjustment of column length
- Gradient program scaling

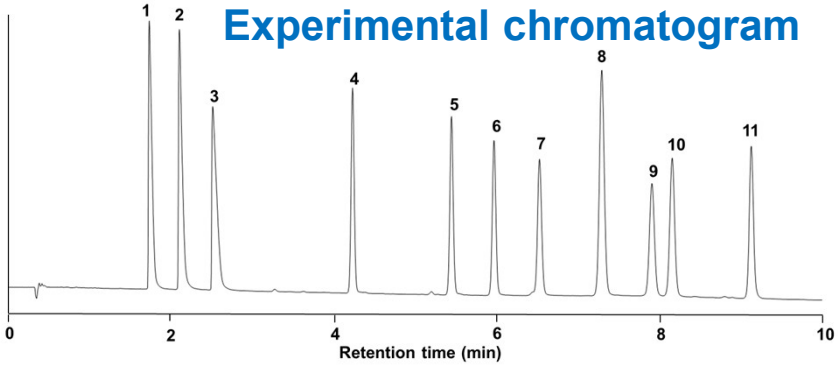
S. Fekete et al. JPBA, 2018, 155, 116-124

T=50 C, t_G=9 min, pH=5.76

Simulated chromatogram



Experimental chromatogram



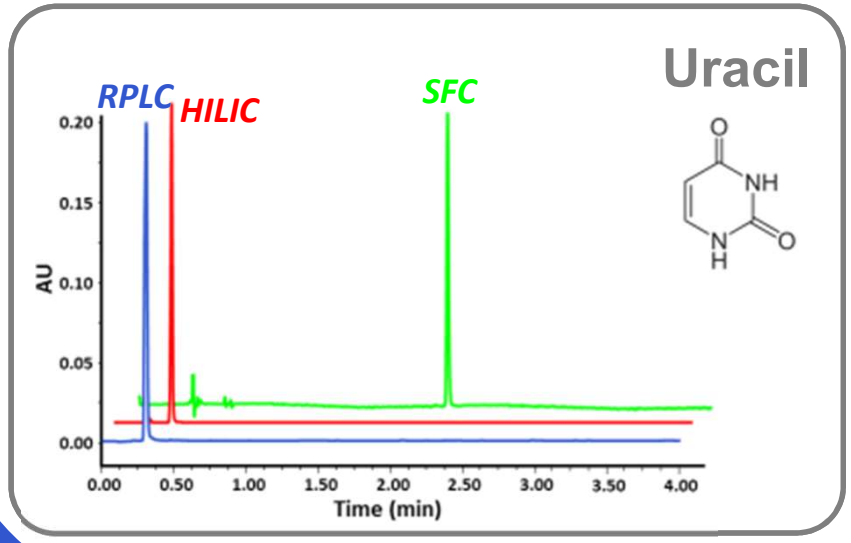
Supercritical fluid chromatography (SFC)

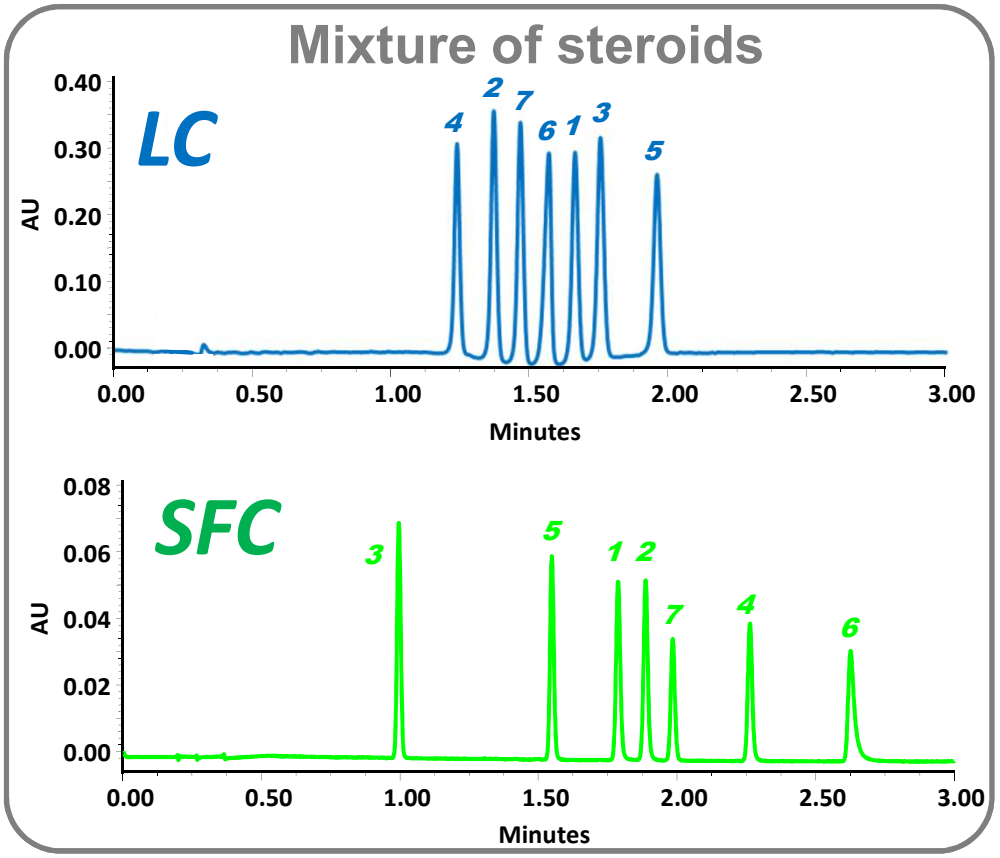
Supercritical state is a hybrid state of matter that can be reached by increasing both temperature and pressure above the critical point.

- ❑ Supercritical fluid viscosity is equivalent to that of a gas
- ❑ Supercritical fluid density is equivalent to that of a liquid

ΔP=74 bar
T=31 C

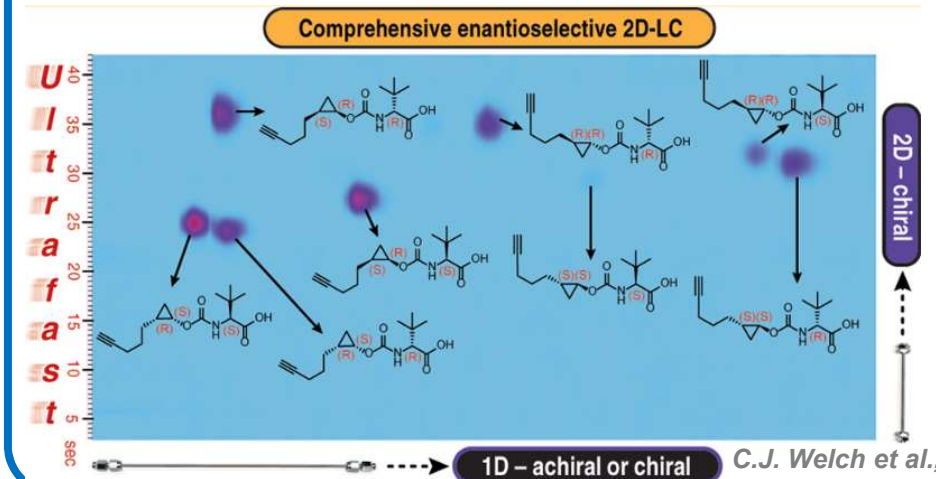
Stationary phase: polar
Mobile phase: mostly CO_2 (apolar) mixed with MeOH (polar)





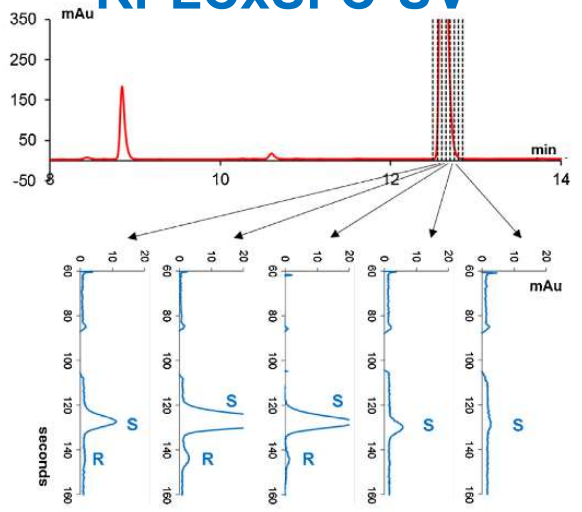
The power of 2D-LC

LCxLC-UV



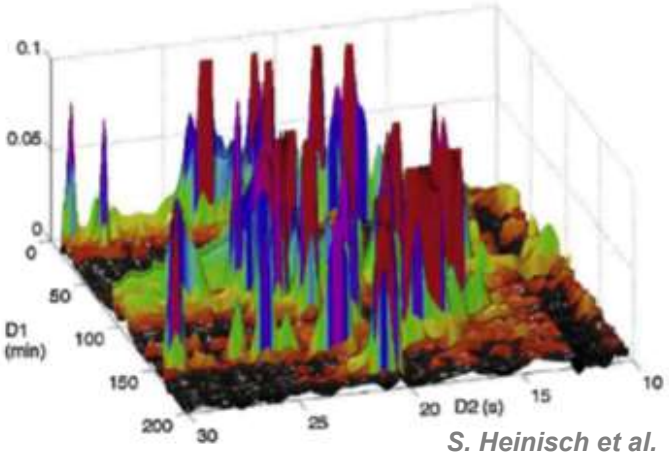
C.J. Welch et al., Merck

RPLCxSFC-UV



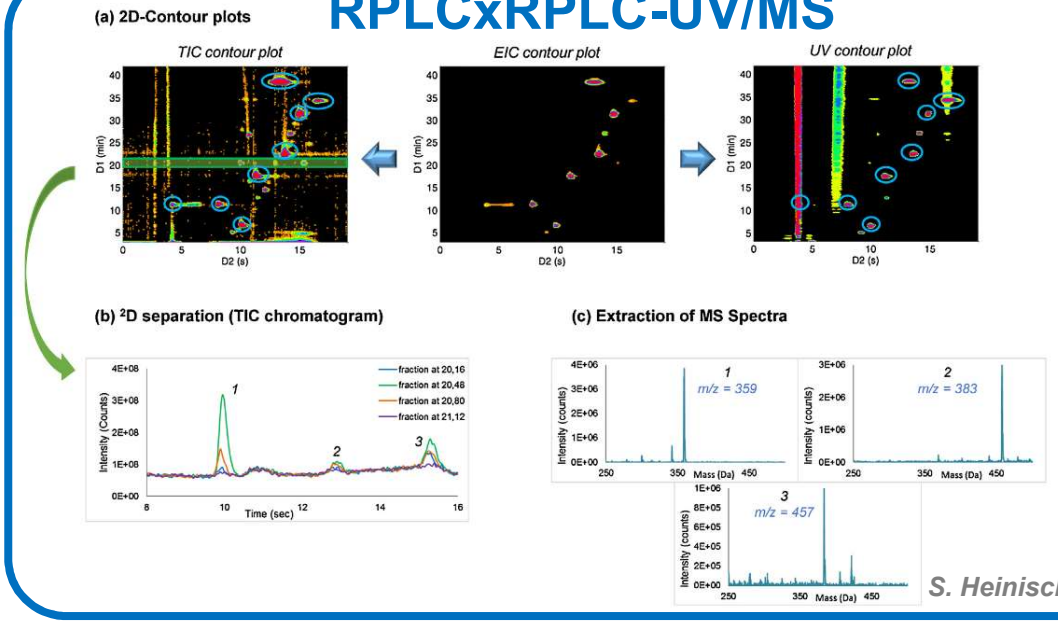
S. Heinisch et al.

RPLCxHILIC of peptides



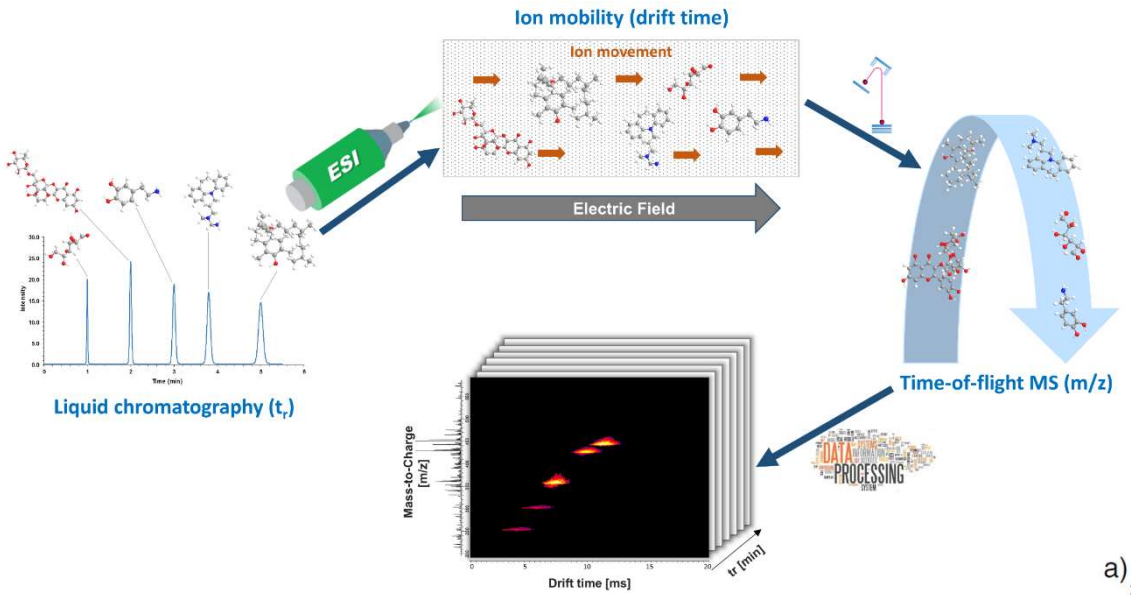
S. Heinisch et al.

RPLCxRPLC-UV/MS



S. Heinisch et al

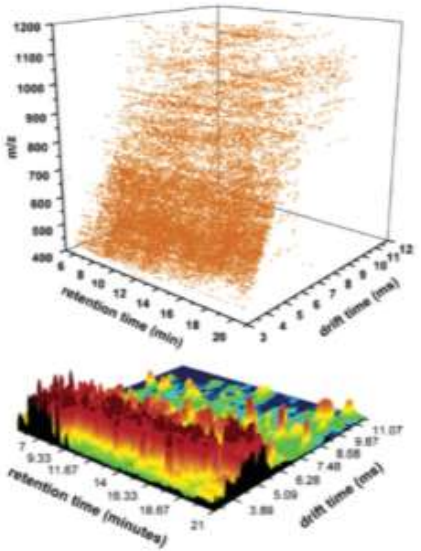
Ion mobility - mass spectrometry (IM-MS)



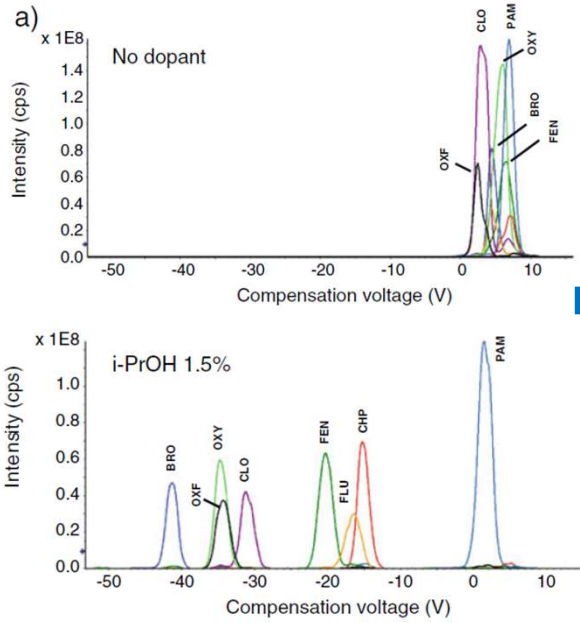
- Compatibility with LC and MS
- High orthogonality with LC
- Highly stable IMS drift times

- Limited peak capacity of IMS
- Expensive/difficult instruments

Plasma proteome



A.C. Hauschild et al., Metabolites, 2, 2012, 733-755



E. Varesio et al., Anal. Bioanal. Chem., 2012, 402, 2555

3. The quest for sensitivity

Genotoxic impurities (ex. nitrosamines) can be found in drug substances and pharmaceutical products.
General impurities should be present at 500 ppm maximum (0.05% level), but genotoxic impurities should be at **1 ppm maximum** (ultra-trace level !).

Cleaning validation is required to establish the cleanliness of manufacturing equipement.
A limit of **10 ppm** of the previously manufactured product is allowed to appear in subsequent products.

In **bioanalysis**, there is a need to achieve very low level of detection **(1 – 10 ppb)** in body fluids.
This is particularly true in therapeutic drug monitoring **(TDM)** and pharmacokinetics **(PK).**

MS as a solution to tackle sensitivity issue?

LC-MS market

Compact single quadrupole MS

MS/MS instruments

High resolution MS

Genotoxic impurities, cleaning validation

Quantitative bioanalysis

Untargeted analysis (-omics)

4. The quest for miniaturization (On-site LC)

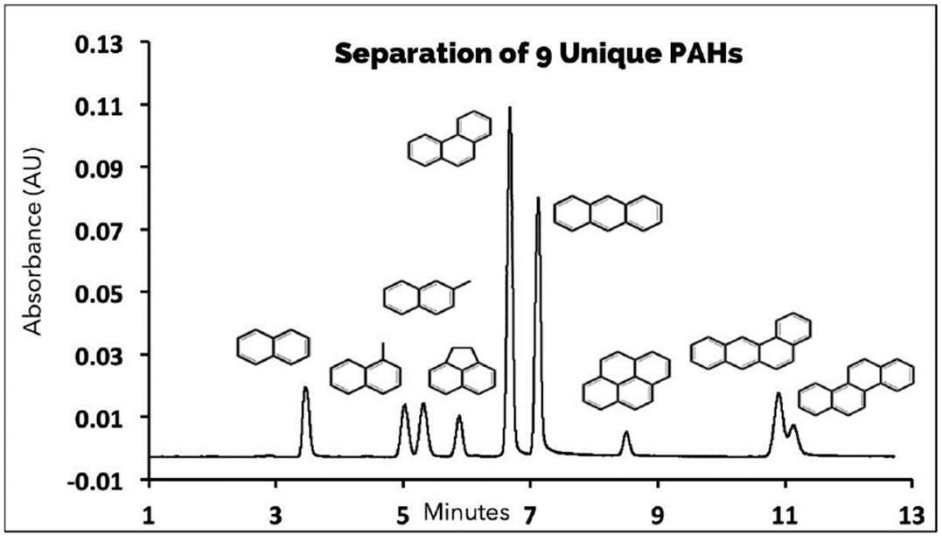
Bronze pittcon excellence award in 2018

Developed in the laboratories of Prof. Milton Lee at Brigham Young University, the Axcend Focus LC opens up new opportunities for analysis as the world's **first truly mobile gradient liquid chromatograph.**

- **Internal battery - portable**
- **Visual output to any web-connected smartphone/tablet**
- **High sensitivity with LED UV-detector**
- **1 week of use only produces a spoon of waste**

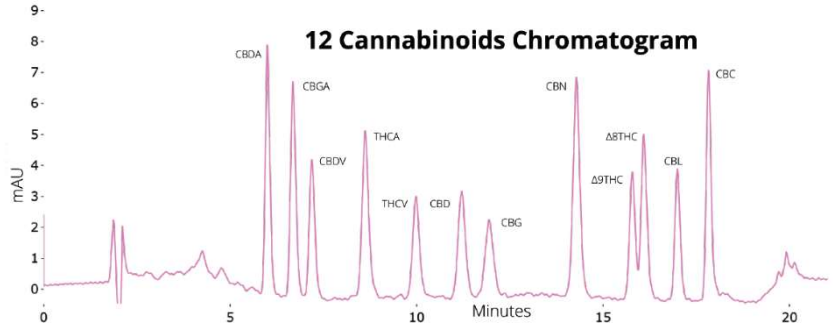
Commercial portable LC system

Application notes with Axcend Focus LC system

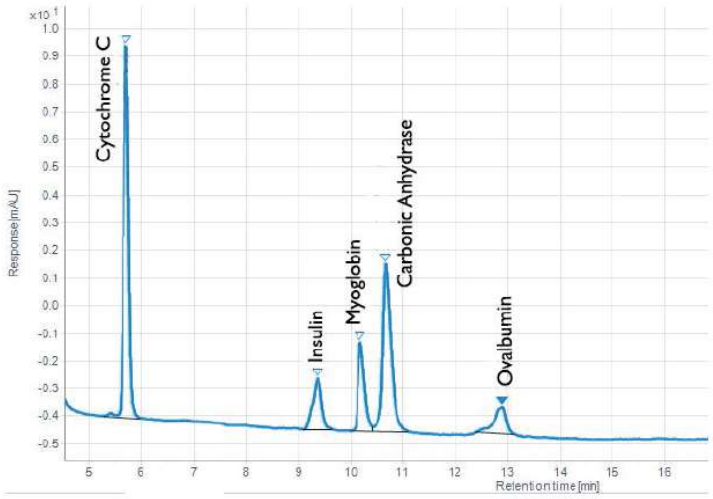


ANALYSIS OF 12 CANNABINOIDS STANDARD

Due to its small size, low-weight, hand-portability, and lower solvent usage, the Axcend Fo
LC is uniquely suited for field and laboratory analysis of cannabinoid compounds. As shov
below, we also detected and identified 12 separate cannabinoids in a Restek standard.



5 PROTEIN TEST COMPOUNDS

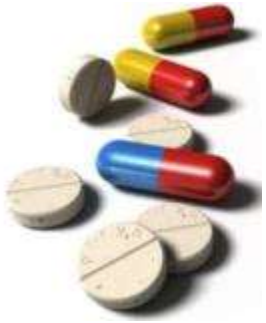


Probably unsuitable for complex analytical problems

Biopharmaceuticals: A new challenge in pharmaceutical analysis ?

Pharmaceutical market in 2004

→ In 2004, only **1** drug among the **TOP 10** can be considered as biopharmaceuticals.

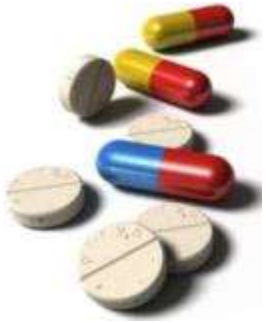


		Market 2004 Sales ($ billion)	Active ingredient	Small molecule/ Biologics	Indication(s)
1	**Sortis**	12	Atorvastatine	Small molecule	Hypercholesterolemia
2	**Zocor**	5.9	Simvastatine	Small molecule	Hypercholesterolemia
3	**Plavix**	5	Clopidogrel	Small molecule	Thrombosis
4	**Nexium**	4.8	Esomeprazole	Small molecule	Ulcer
5	**Zyprexa**	4.8	Olanzapine	Small molecule	Neuroleptic
6	**Norvasc**	4.8	Amlodipine	Small molecule	Hypertension
7	**Seretide**	4.7	Salmeterol	Small molecule	Bronchodilator
8	**Erypo**	4	Alpha-Epoetin	Protein	Anemia
9	**Ogastro**	3.8	Lansoprazole	Small molecule	Ulcer
10	**Effexor**	3.7	Venlafaxine	Small molecule	Antidepressive

IMS Health, 2005, Volume 83, Number 36, pp. 49-53

Pharmaceutical market in 2017

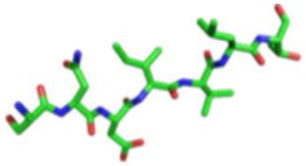
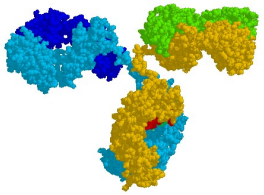
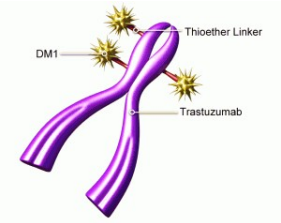
→ In 2017, **7** drugs among the **TOP 10** can be considered as biopharmaceutics (mAbs, fusion proteins).



		Market 2017 Sales ($ billion)	Active ingredient	Small molecule/ Biologics	Indication(s)
1	**Humira**	18.4	Adalimumab	Monoclonal Antibody	Rheumatoid arthritis
2	**Rituxan**	9.2	Rituximab	Monoclonal Antibody	Various cancers
3	**Revlimid**	8.1	Lenalidomide	Small molecule	Various cancers
4	**Enbrel**	7.8	Etanercept	Fusion protein	Rheumatoid arthritis
5	**Herceptin**	7.4	Trastuzumab	Monoclonal Antibody	Breast cancers
6	**Eliquis**	7.3	Apixaban	Small molecule	Anticoagulant
7	**Remicade**	7.1	Infliximab	Monoclonal Antibody	Rheumatoid arthritis
8	**Avastin**	7.0	Bevacizumab	Monoclonal Antibody	Various cancers
9	**Xarelto**	6.5	Rivaroxaban	Small molecule	Anticoagulant
10	**Eylea**	6.0	Aflibercept	Fusion protein	Macular degeneration

https://www.genengnews.com/the-lists/the-top-15-best-selling-drugs-of-2017/77901068?page=1

Analytical characterization of molecules

Small molecules	Peptides	Proteins	mAbs	ADC
				
salicylic acid MW 138 Da	triptorelin MW 1,311 Da	human growth hormone MW 22,125 Da	Rituximab MW ~150,000 Da	Brenduximab-vedotin MW ~150,000 Da

Analytical complexity

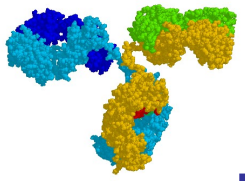


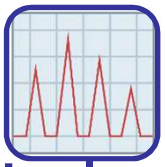


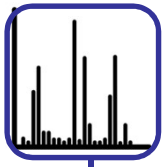
Important micro-heterogeneity during the production of biologics

Analytical challenge !

Evolution of analytical toolbox for proteins







	Electrophoresis 1990's	Chromatography 2000's	LC-MS and 2D-LC 2010's
Primary sequence	AA analysis MALDI-TOF	AA analysis **LC**-ESI-MS/MS	AA analysis UHPLC-**ESI-HRMS** 2D-LC-**ESI-MS**
Size variants	SEC **SDS-PAGE**	**SEC** CGE	UHP-SEC **(MS)** CGE A4F
Charge variants	**IEF** IEX	cIEF **IEX**	icIEF CZE Modern IEX **(MS)**
Glycan analysis	**CZE**-LIF (APTS)	CZE-LIF (APTS) **HILIC**-FD (2-AB)	CE-LIF **(MS)** (APTS) HILIC-**MS** (Rapidfluor) MALDI-**TOF/MS**
High order structure	Calorimetry (DSC)	CD Fluorescence	HDX-**MS** Near and far-UV CD NMR

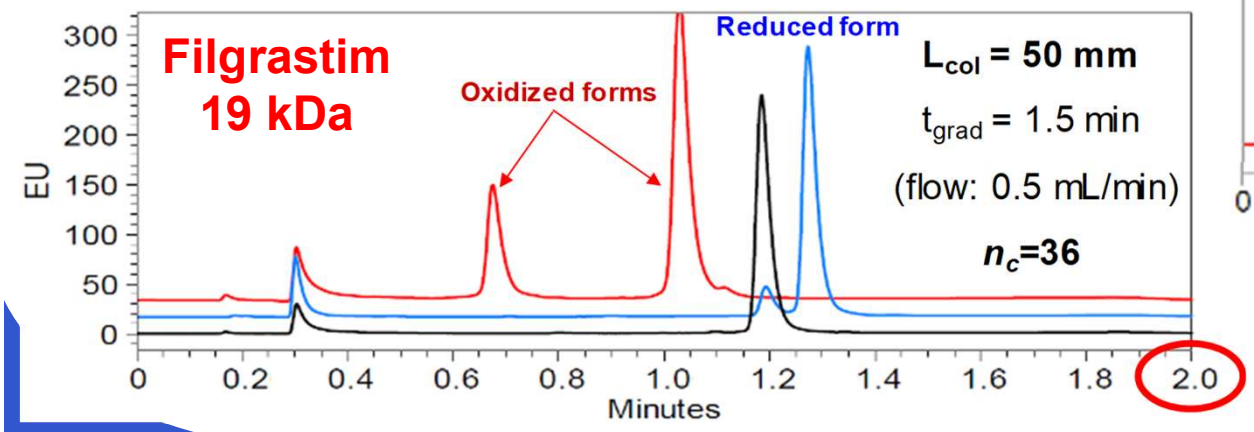
RPLC of intact proteins – possible?

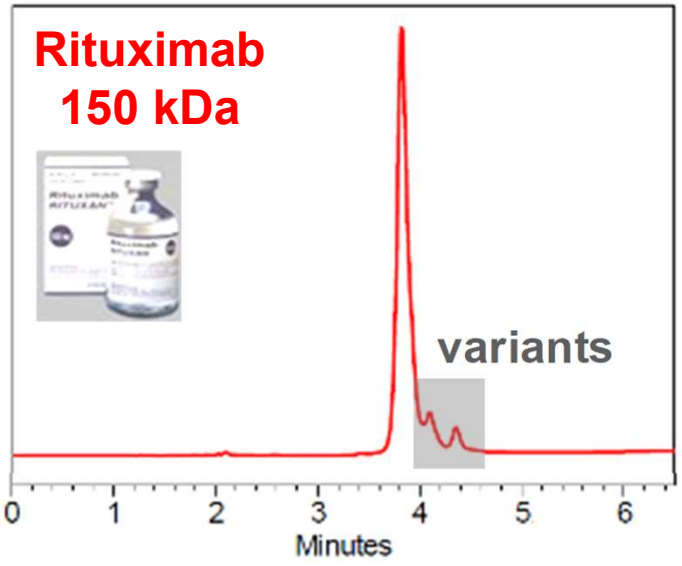
For a long time, proteins were only analyzed in RPLC at the peptide level. Thanks to the numerous technological progresses, RPLC can now also be used at the intact protein level.

What are the challenges associated with RPLC of proteins?

- Limited kinetic performance
- Poor recovery
- Ion pairing dependence
- Reliance on high temperature

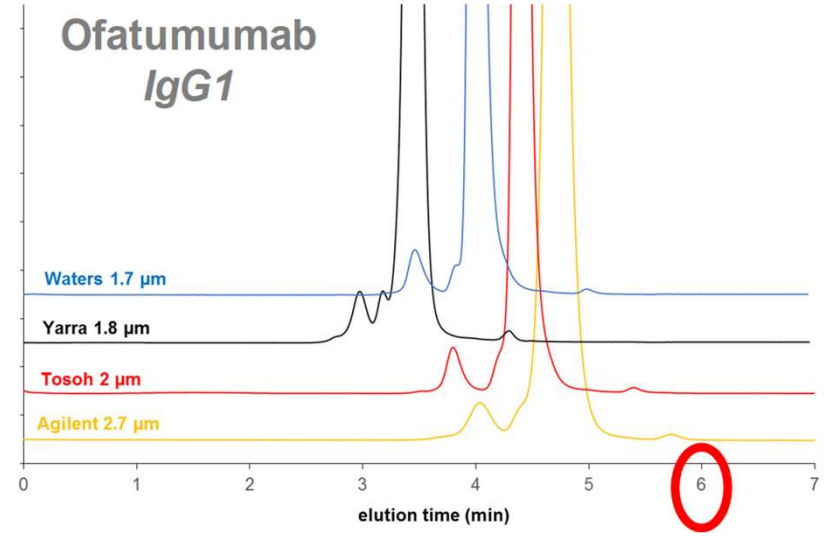
- Core-shell particles
- Sub-2µm particles
- Large pore-size
- Temperature stability
- Optimized chemistry

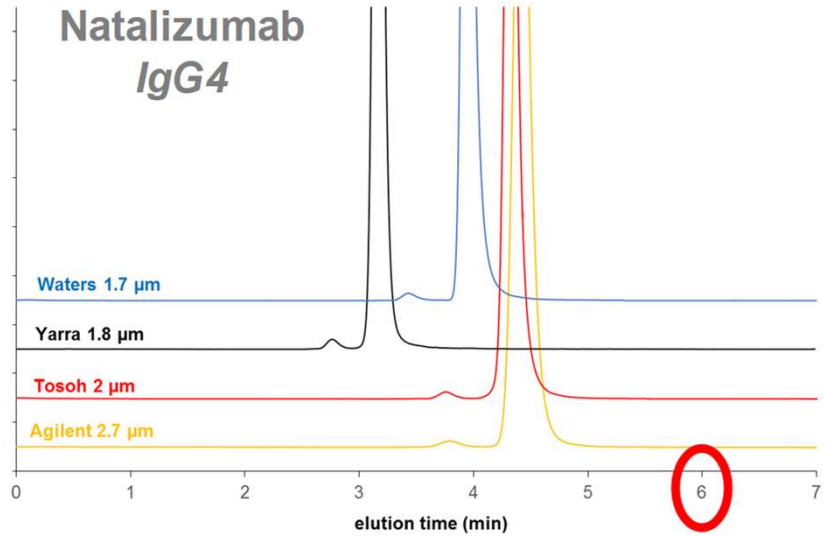


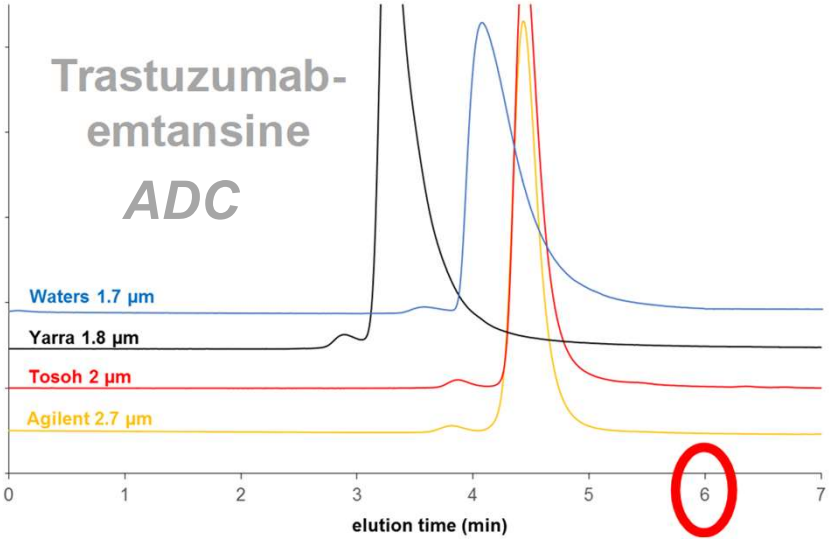


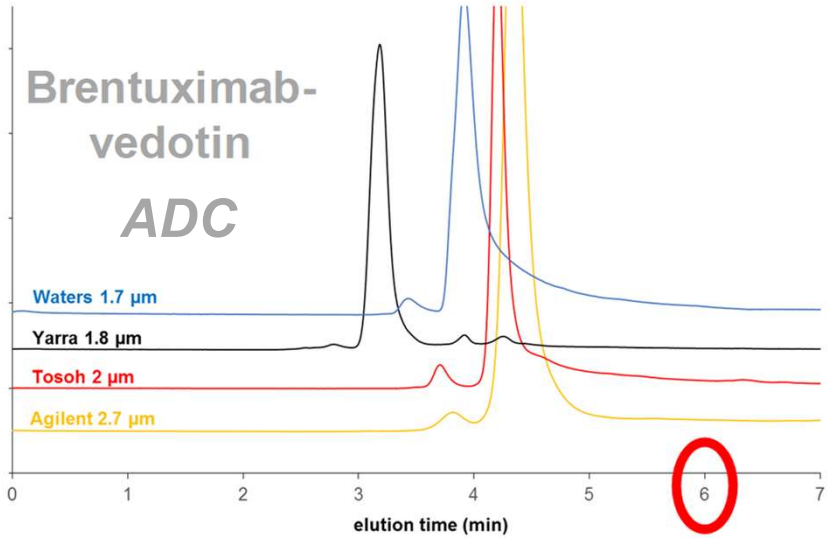
Modern SEC: using sub-3µm columns

Mobile phase: 100 mM disodium hydrogen-phosphate buffer and 200 mM sodium chloride in water, pH 6.8 at 25°C and columns were operated *at 350 µL/min*





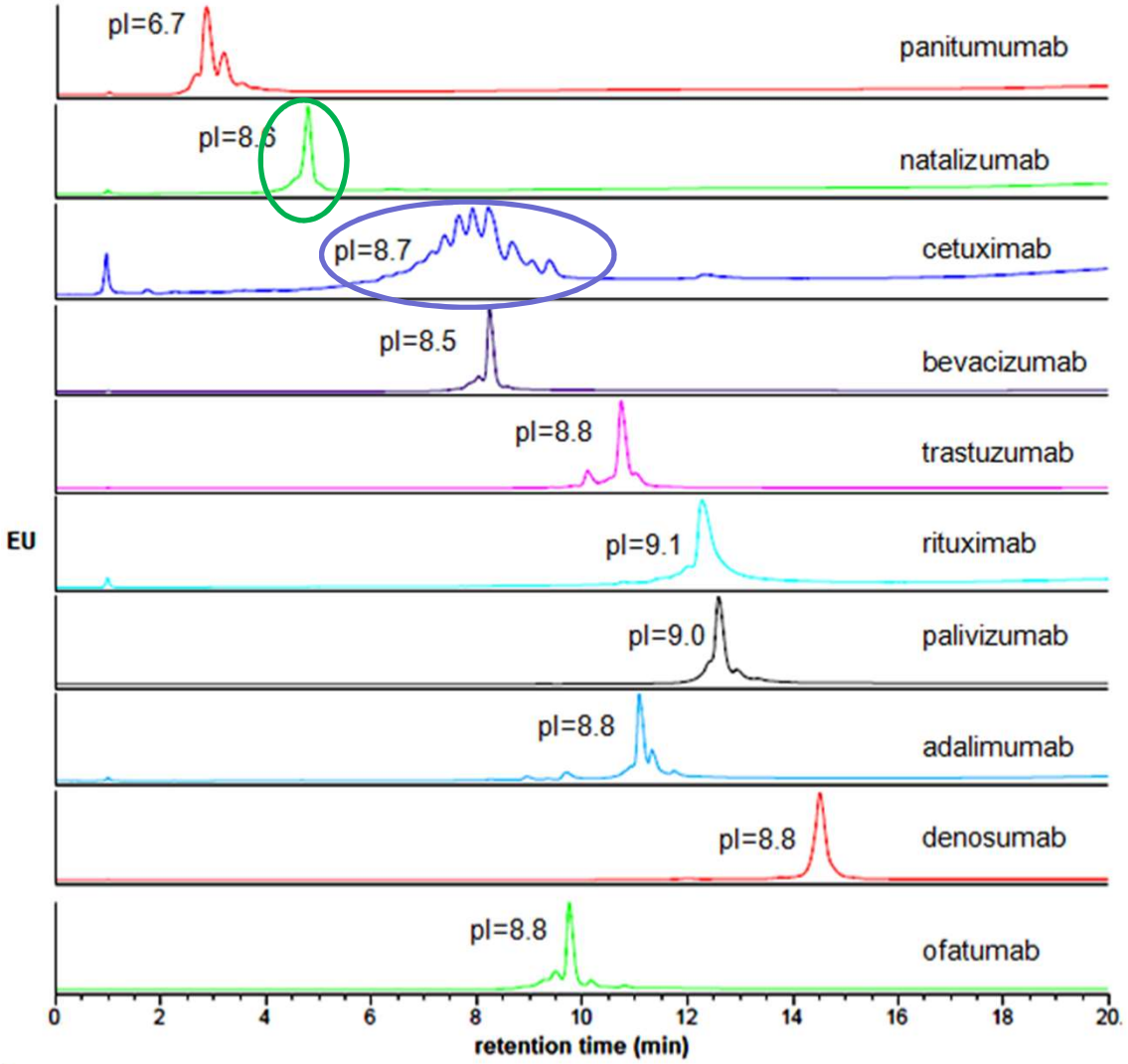




Modern SEC columns: faster analysis, good chemical inertness

pH-gradient IEX: an alternative for mAbs?

Generic pH-gradient method for the caracterisation of 10 intact mAbs



- Column: YMC BioPro SP-F (100 x 4.6 mm).
- Mobile phase: “A” Thermo CX-1 buffer **pH=5.6**, “B” Thermo CX-1 buffer **pH=10.2**
- Gradient: 0 – 100 %B in 20 min
- Flow rate: 0.6 mL/min
- Temperature: 30 ºC
- Detection: FL (280 – 360 nm)

Similar performance in salt and pH gradient.

No need to manually prepare buffers, interesting solution for the industry.

S. Fekete et al., J. Pharm. Biomed. Anal., 2015, 102, 282-289

Conclusion and perspectives

From an analytical point of view, continuous need for:

- Speed / Resolution
- Selectivity
- Sensitivity
- Miniaturization/simplification

Increasing complexity of pharmaceutical products:

- Mixture of several API (i.e. HIV treatment)
- API with multiple chiral centers
- Difficult formulation (surfactants, lipids, cyclodextrins…) to address low drug solubility
- Biopharmaceuticals + biosimilars + new mAb formats
- Oligonucleotides, cell and gene therapy

Main bottleneck today:

- Data treatment
- Regulatory aspects are more and more strict

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Possible alternative to chromatography:

- Spectroscopy (RAMAN, NIR)