

2D-LC-MS: Transitioning from Research Laboratories to Main Steam Pharmaceutical Analysis

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HPLC 2014, New Orleans, USA

2D-LC-MS in Small Molecule Pharmaceuticals – *why?*

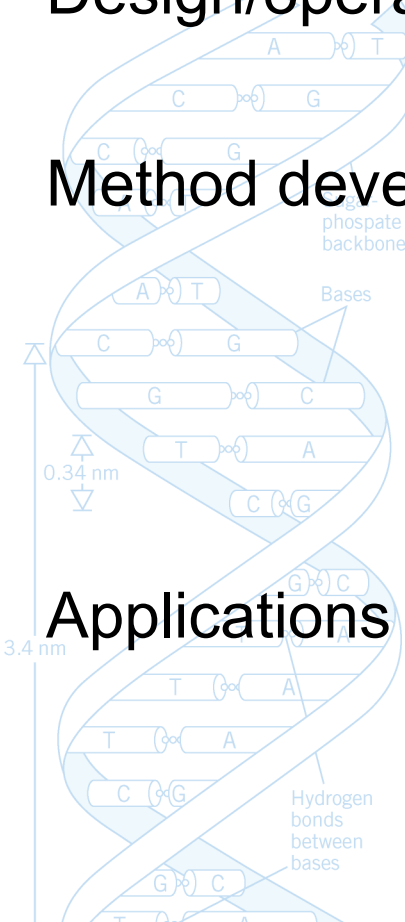
Design/operation of a 2D-LC system

Method development strategy

Comprehensive v/s heart-cutting

Stationary phase selection strategy

Applications of 2D-LC in Small Molecule Pharmaceuticals



Highly regulated industry

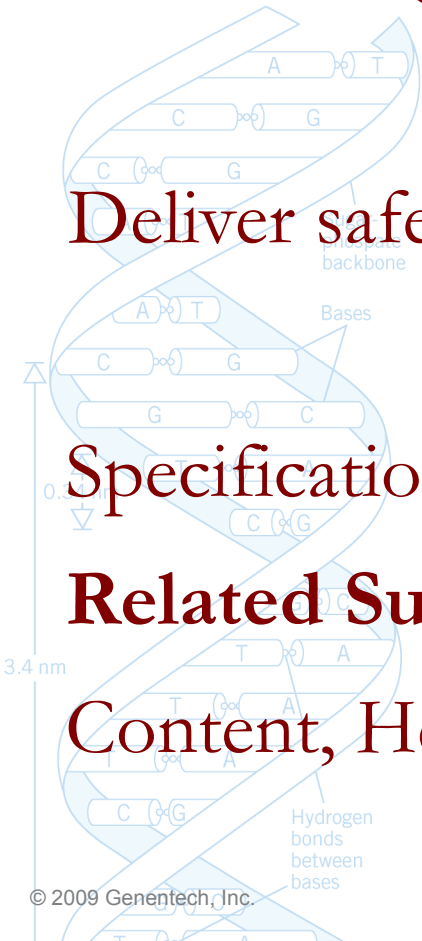
Oversight from regulatory agencies around the world

Deliver safe and efficacious medications

Specifications: Appearance, Identity, **Assay and**

Related Substances, Residual Solvents, Water

Content, Heavy Metals and Specified Metals etc



ICH impurities (Q3A):

Reporting threshold $> 0.05\%$

Identification threshold $\geq 0.10\%$

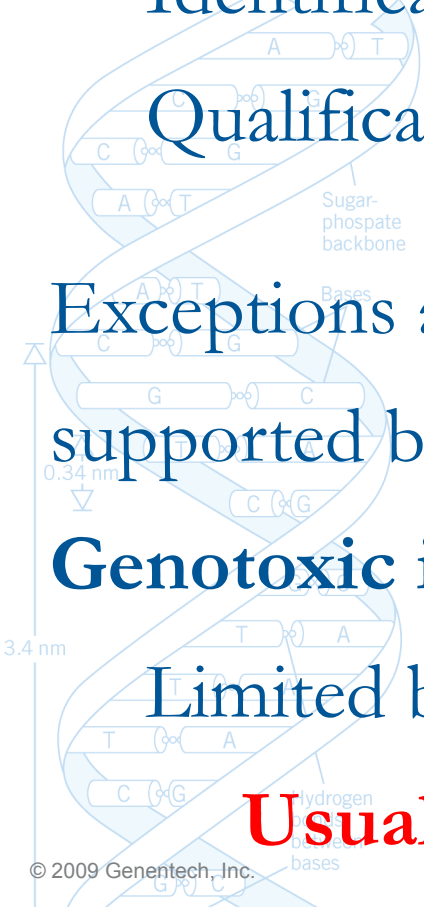
Qualification threshold $\geq 0.15\%$

Exceptions are impurities with tox coverage, metabolites, supported by manufacturing capabilities

Genotoxic impurities (M7):

Limited by max daily dose & duration of exposure

Usually low parts per million



Column screening at different pH's, peak tracking

Demonstrate specificity - SM's, intermediates, potential imp

Demonstrate stability indicating capability of method with stressed samples - acid, base, peroxide, heat, humidity, light

Relies on DAD and MS for detection - limiting factor

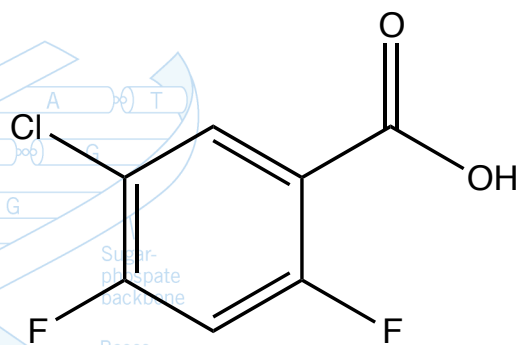
Peaks eluting around main component have similar

UV spectra - Limits DAD

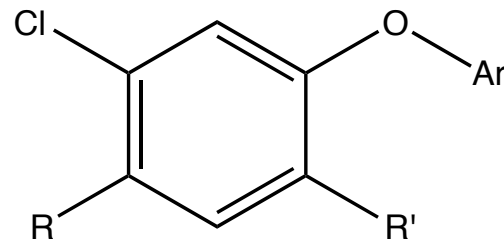
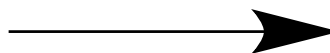
Isomers - Limits MS

Potential Solution: Two-dimensional Chromatography

Synthetic Scheme of API (Hypothetical)

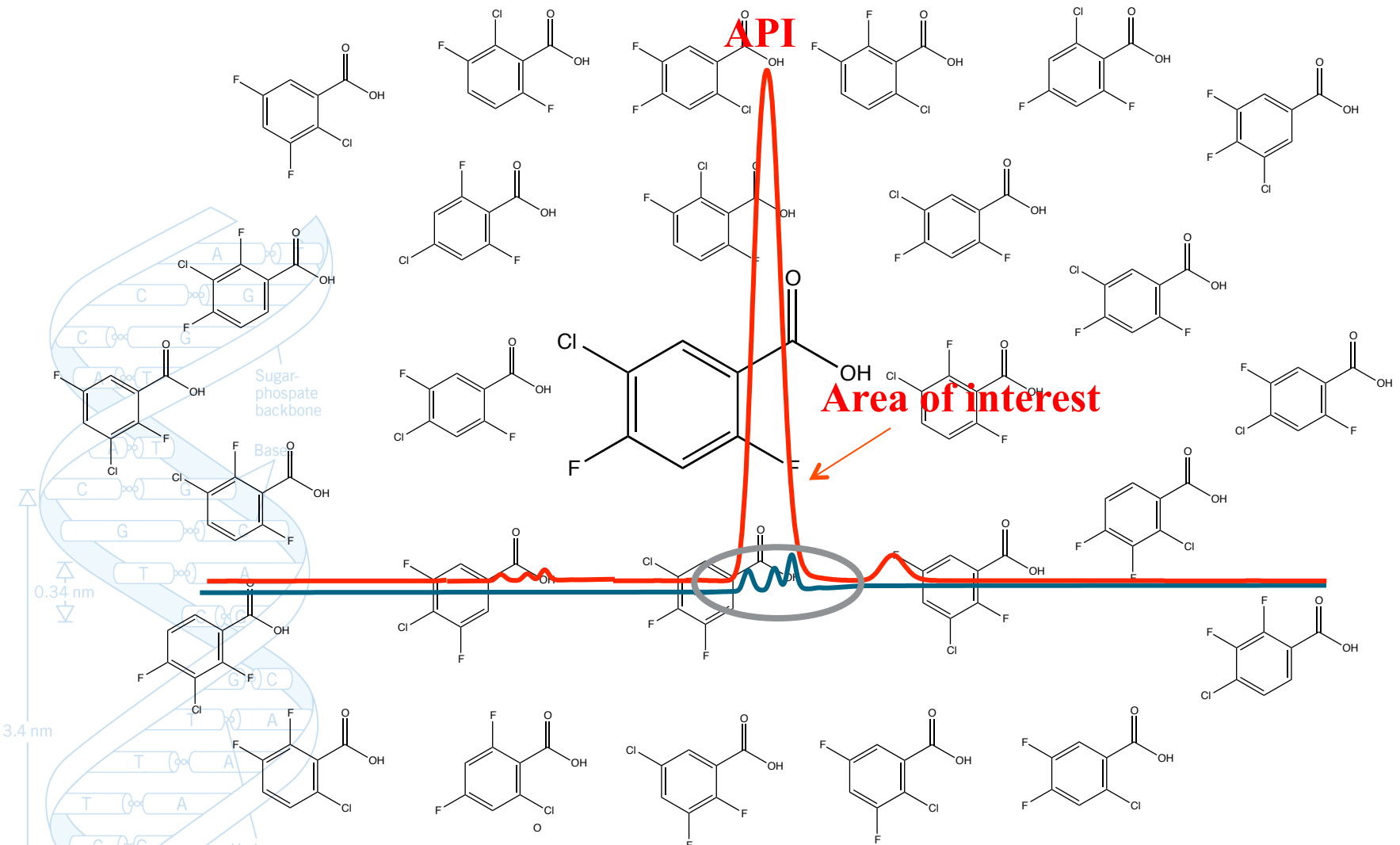


5-chloro-2,4-difluorobenzoic acid
Chemical Formula: $C_7H_3ClF_2O_2$
Molecular Weight: 192.55

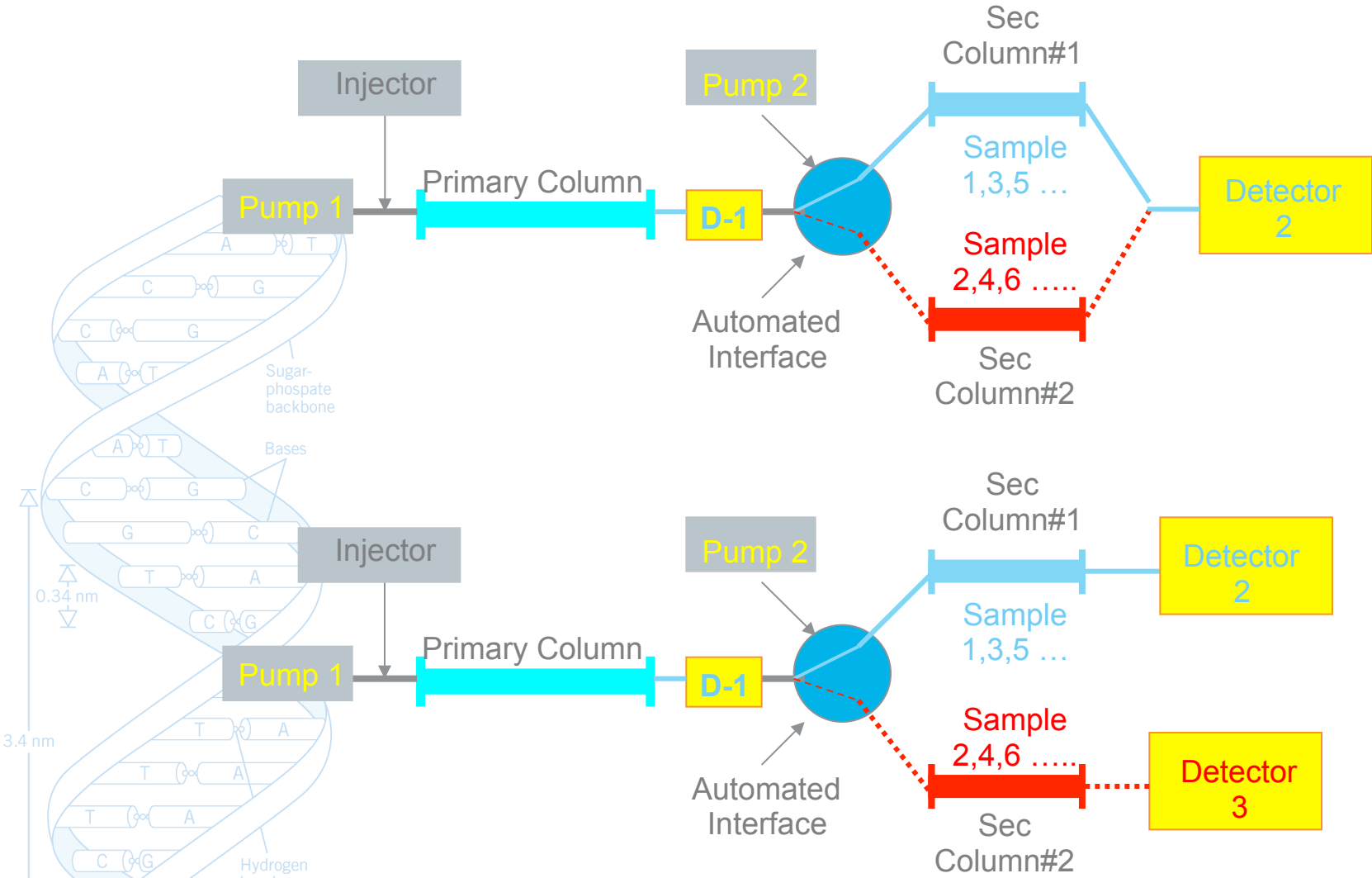


Active pharmaceutical ingredient (API)
Chemical Formula: $C_xH_yClF_2O$

Potential Isomers / Impurities in SM

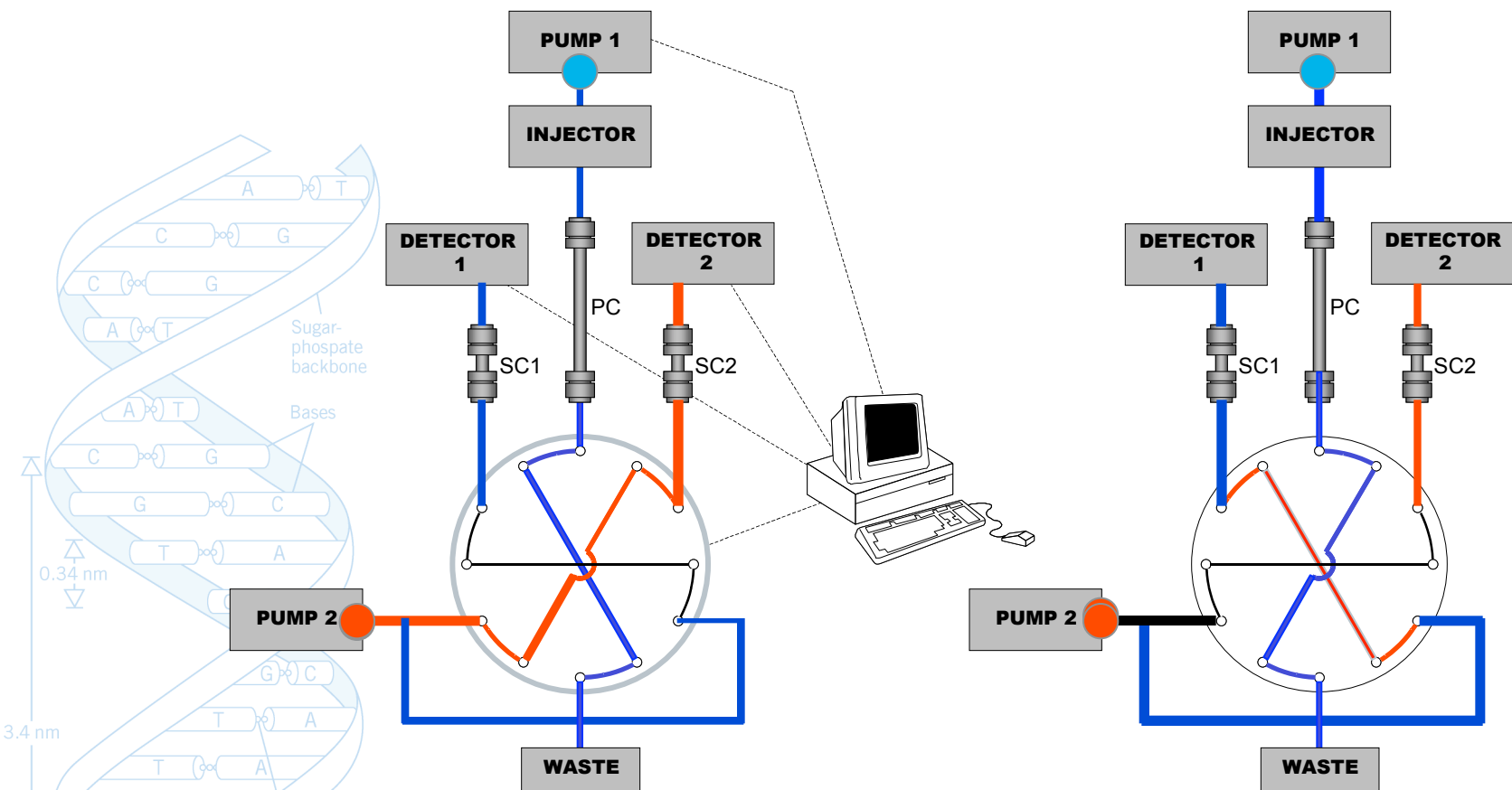


Configuration of LCxLC

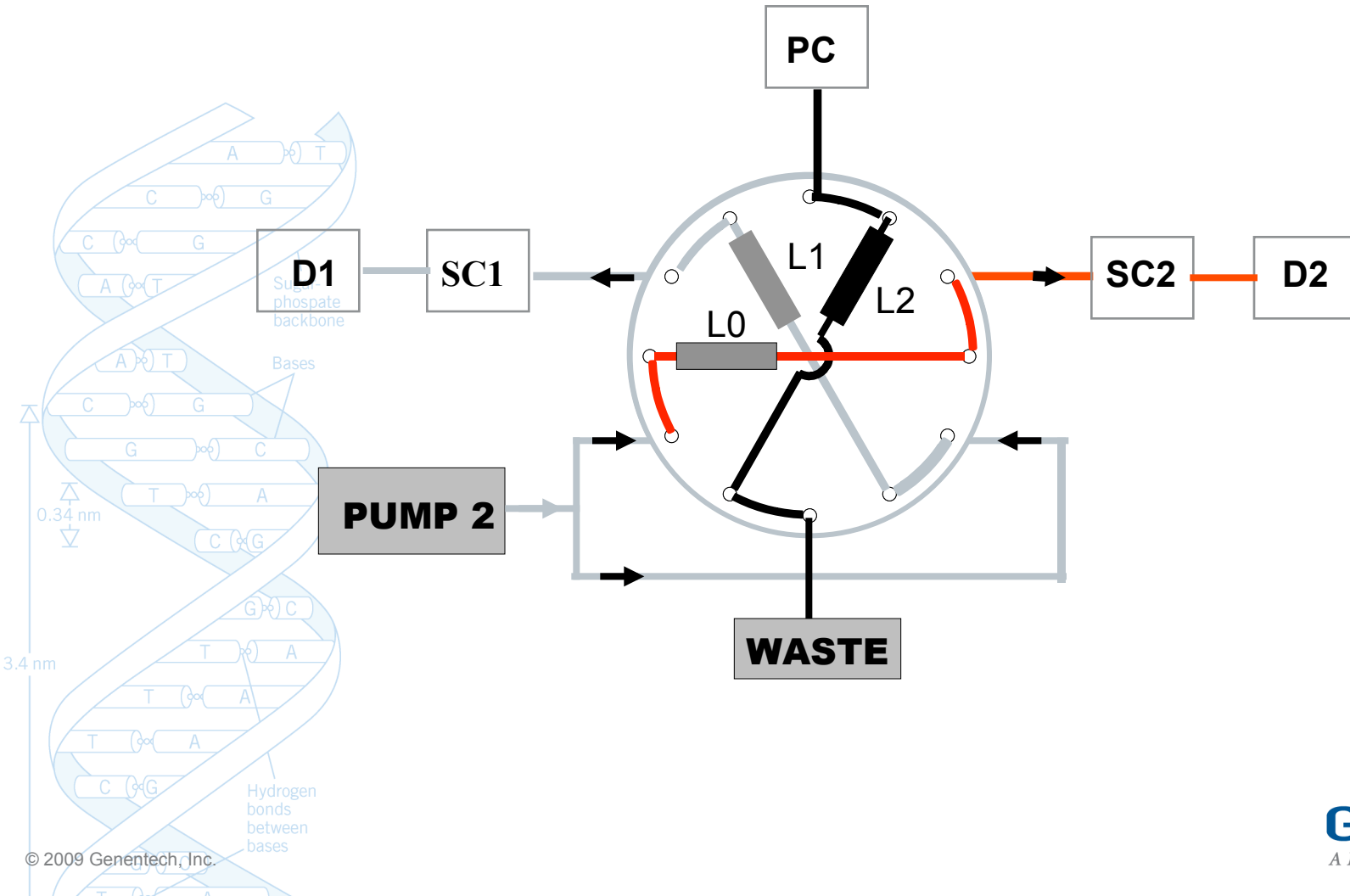


Position 1

Position 2



Venkatramani, C. J., Zelechonok, Y., Anal. Chem. 2003, 75, 3484–3494.

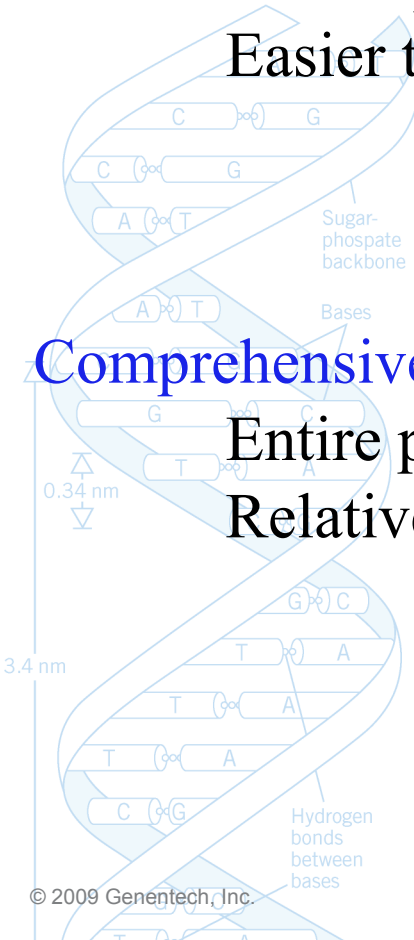


Heart-Cutting 2D-LC

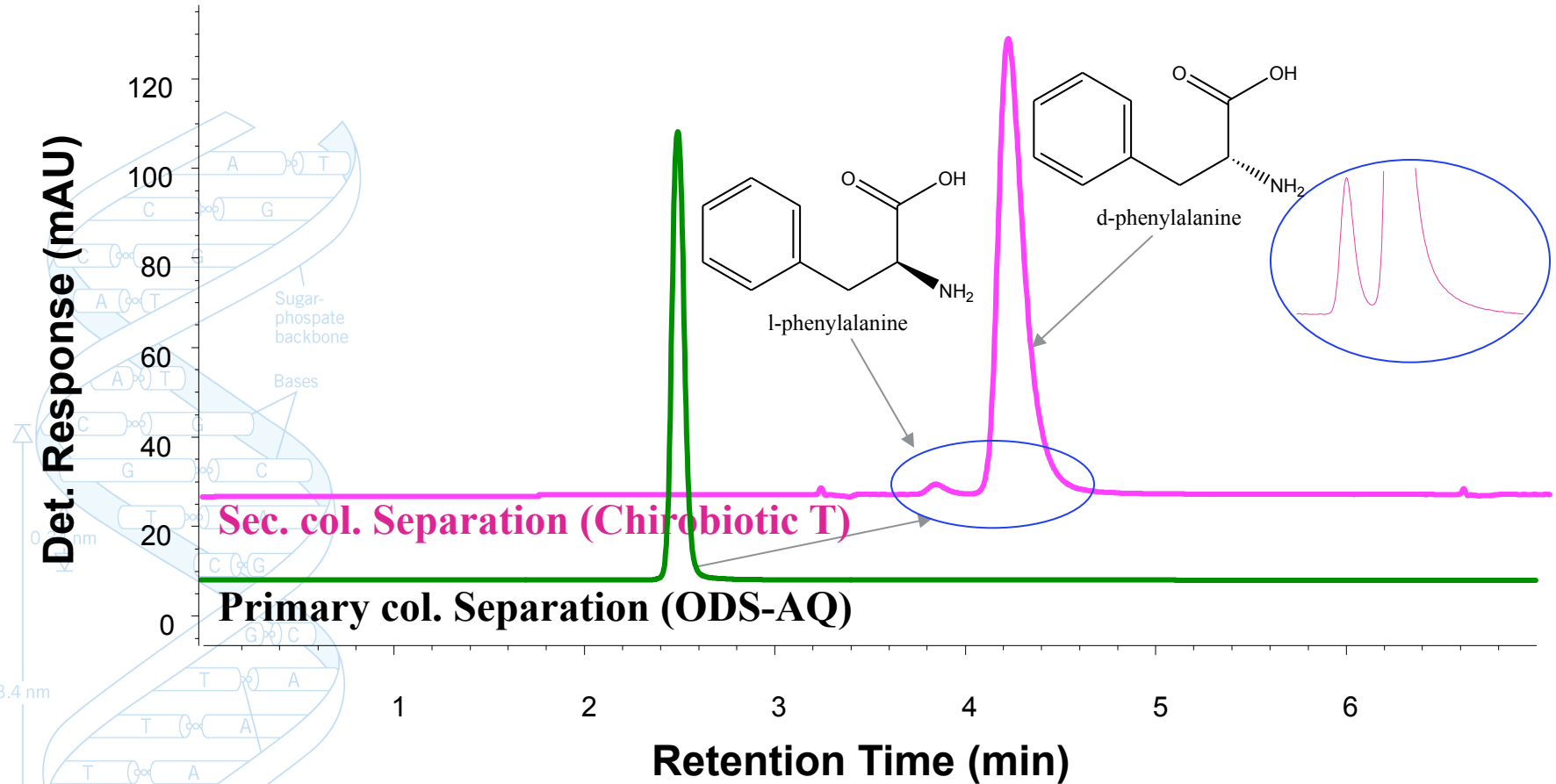
Part of primary column eluent sampled into secondary column
Easier to design and adequate – camera

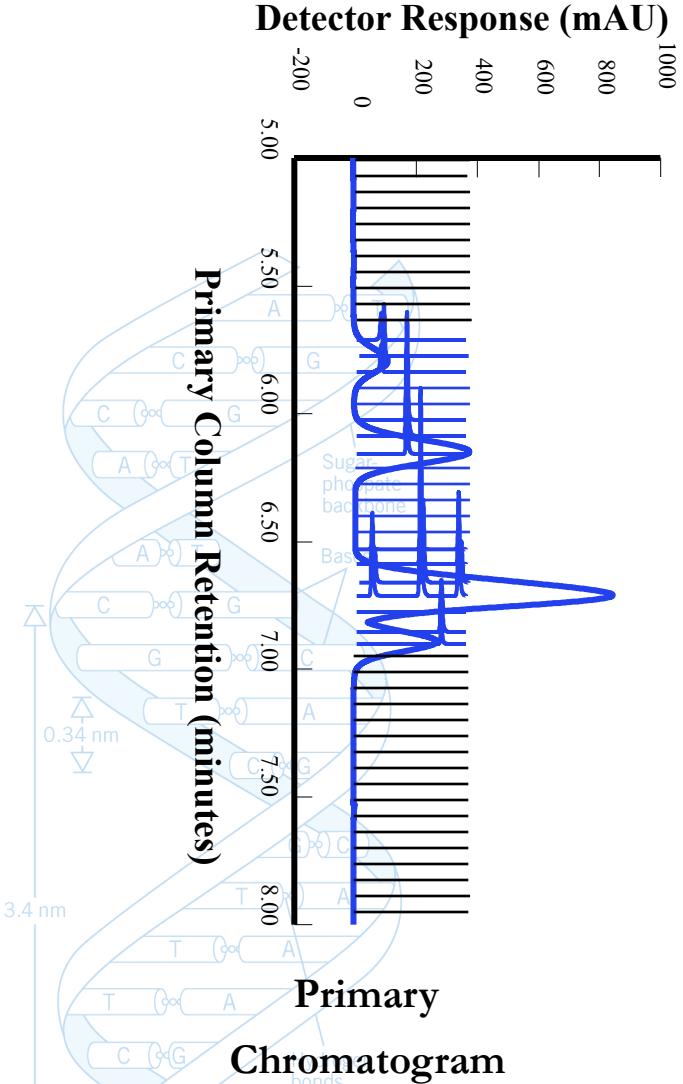
Comprehensive 2D-LC

Entire primary column eluent sampled into secondary column
Relatively difficult and detailed - camcorder

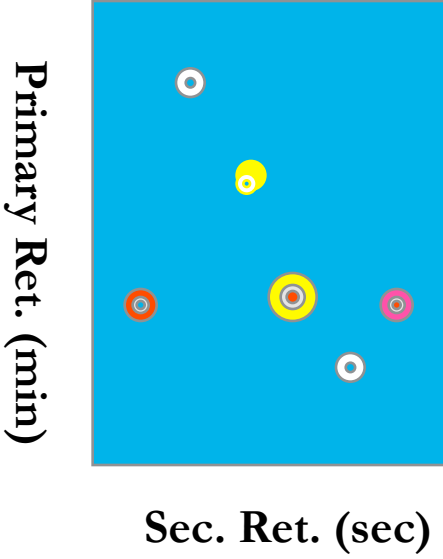


Simultaneous achiral-chiral separation – heart-cutting





Secondary Chromatograms



Primary column:

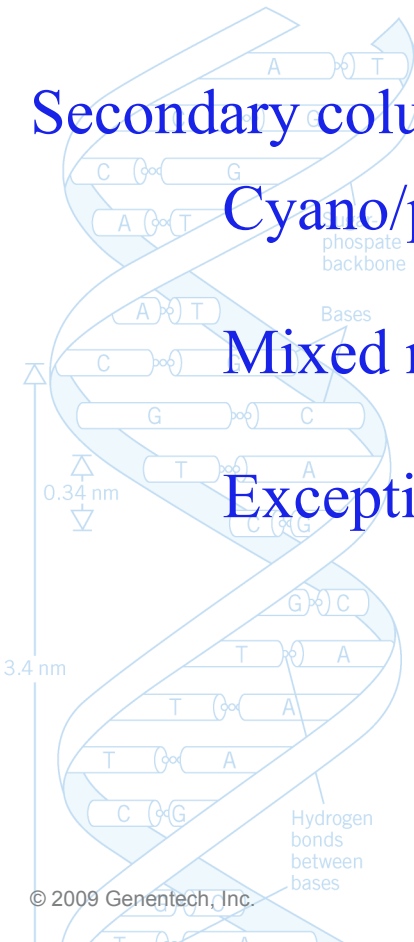
Typically C18 & C8, occasionally phenyl and cyano phase

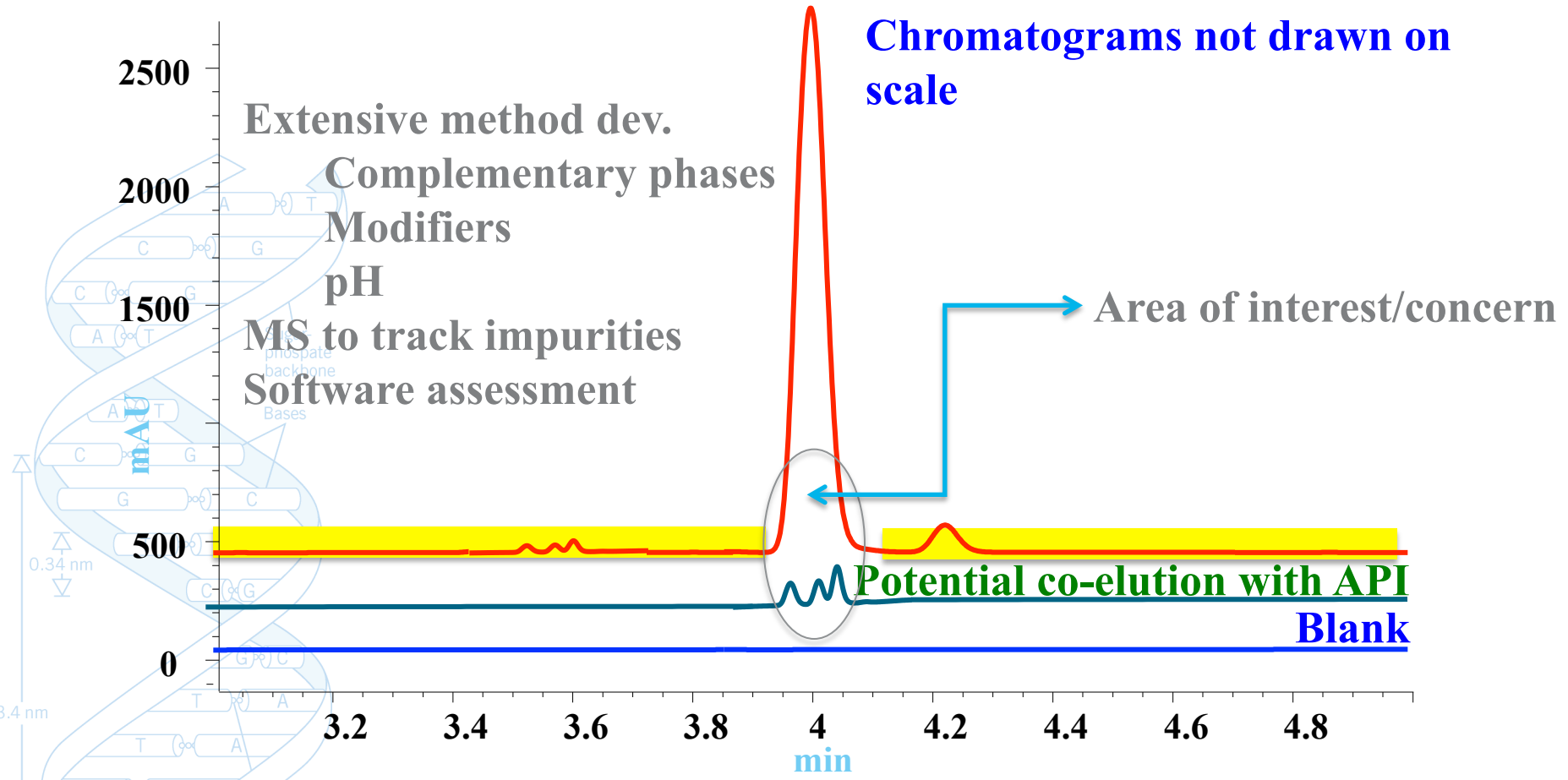
Secondary column

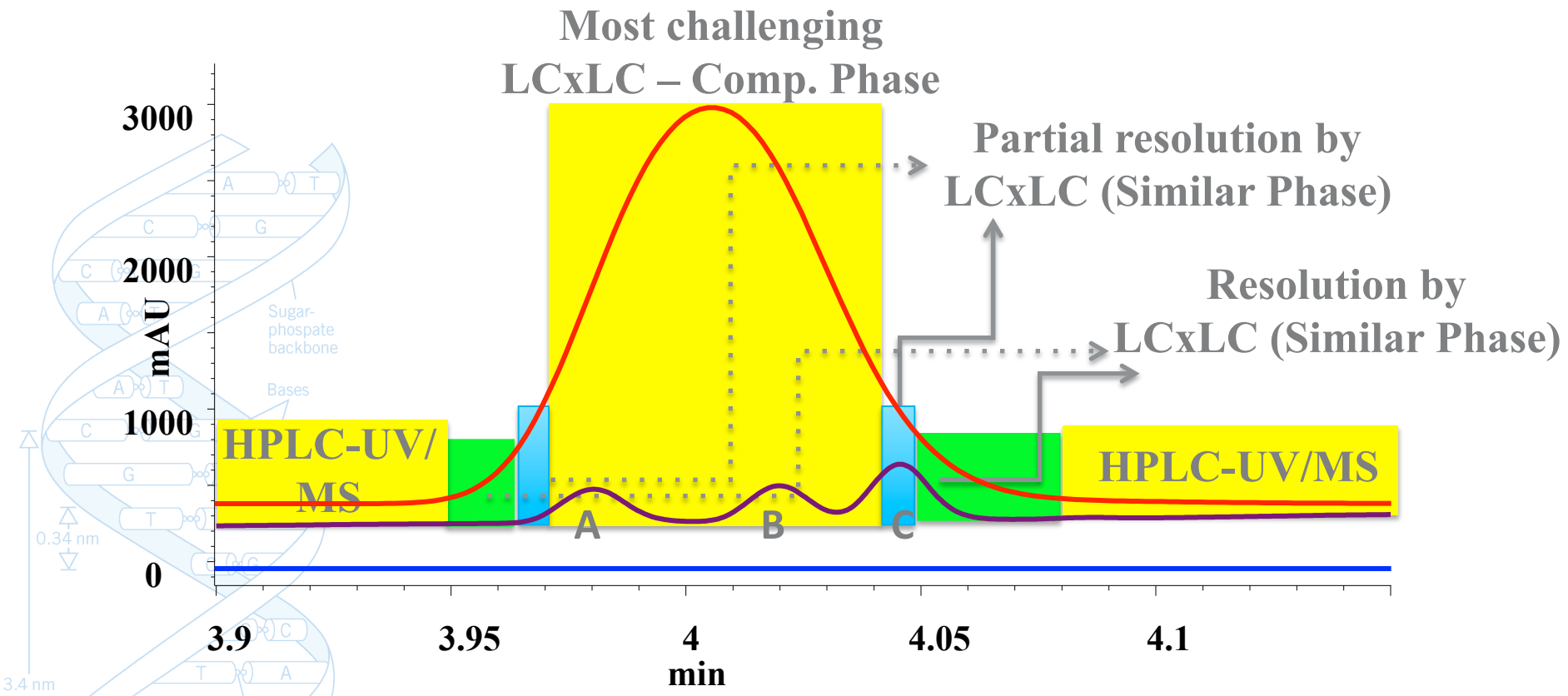
Cyano/phenyl complementary to C18 and C8

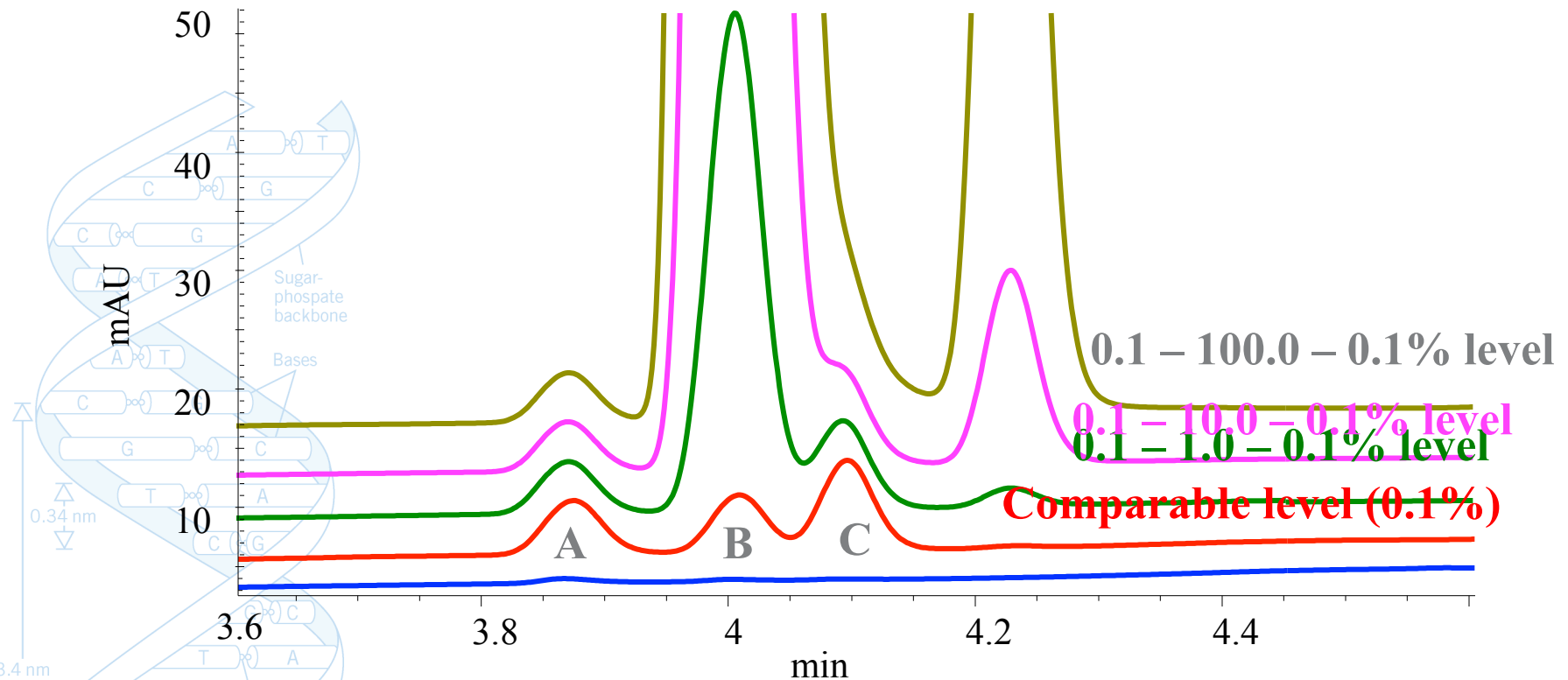
Mixed mode phases

Exception: Similar phase – select region of chromatogram

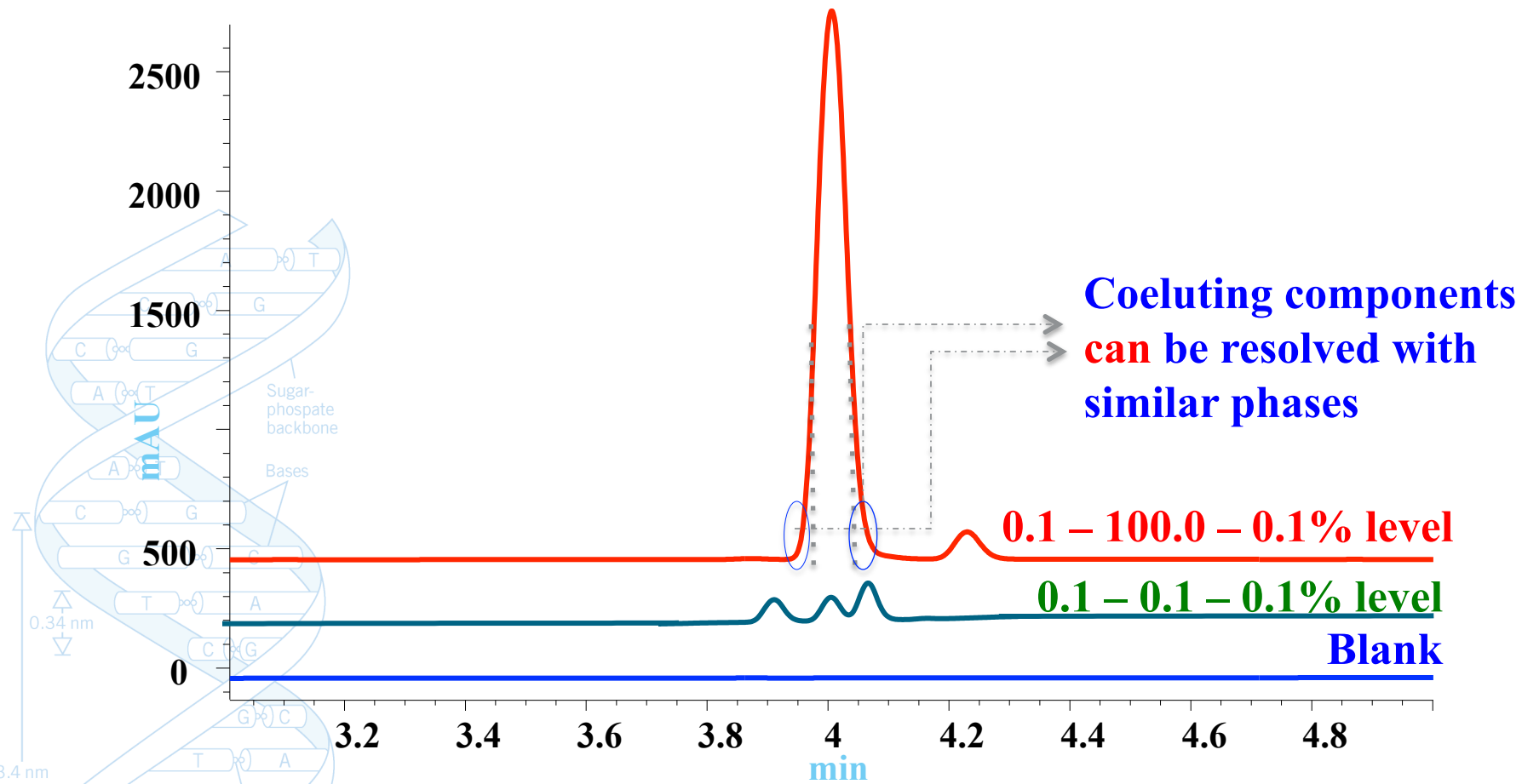




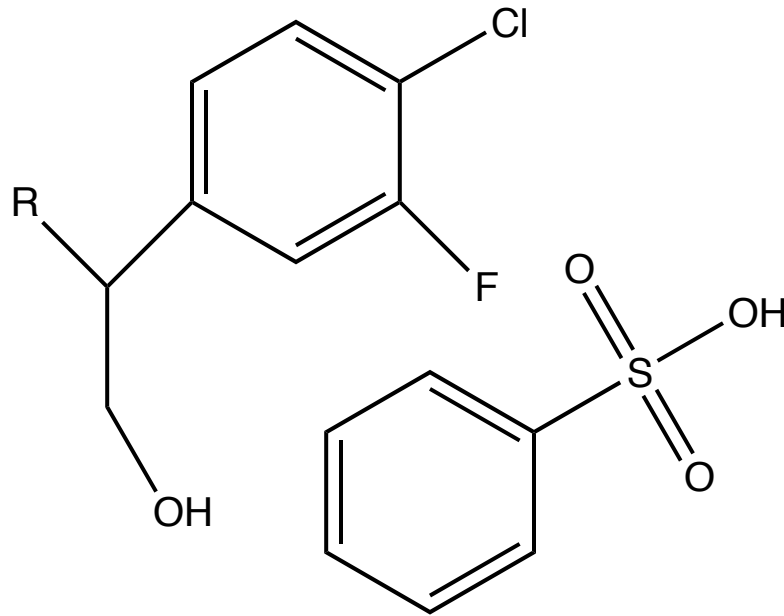




At nominal concentration of main component, resolution between main component and impurity peaks in the proximity is lost

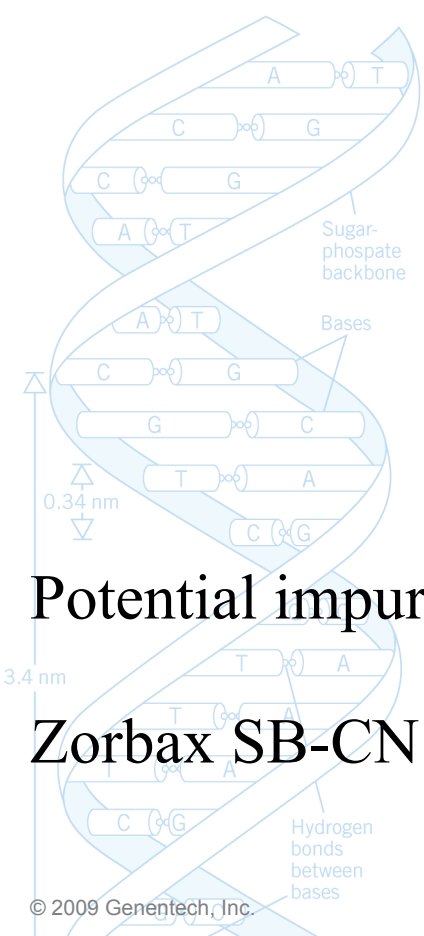


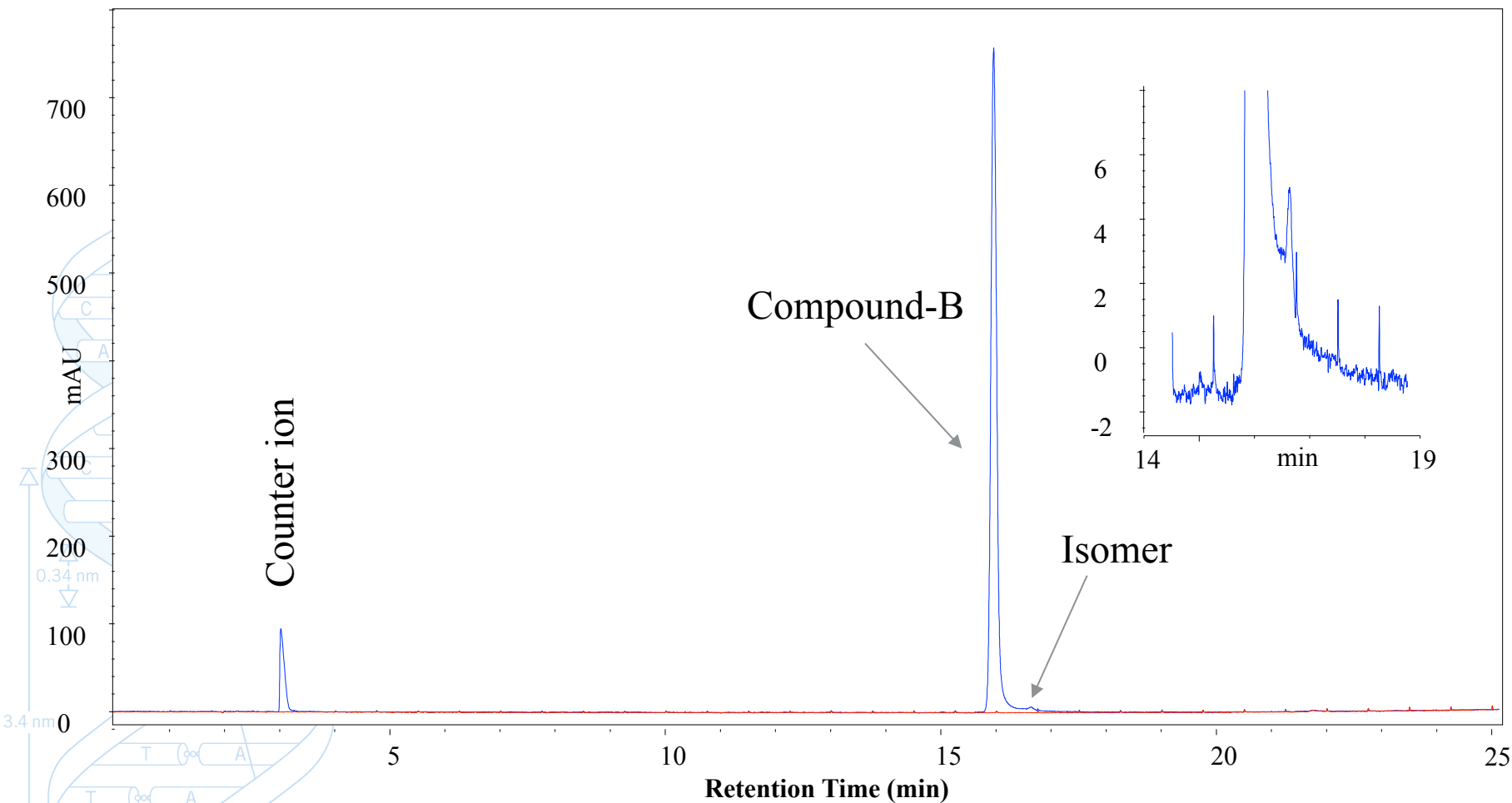
Similar phase limited to selected regions of primary column separation

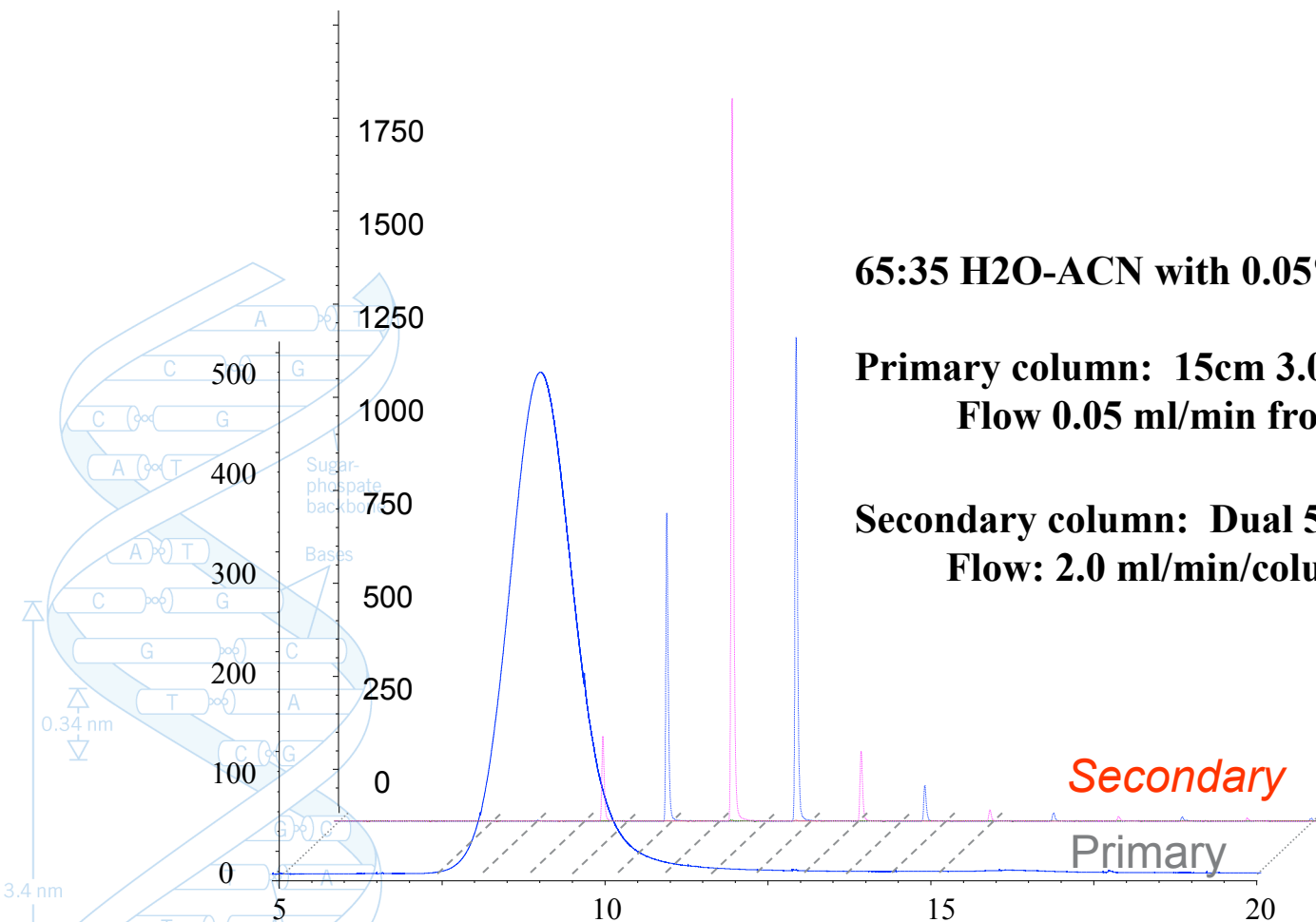


Potential impurity, an isomer of API, difficult to resolve

Zorbax SB-CN column ~ shallow step gradient







65:35 H₂O-ACN with 0.05% H₃PO₄

**Primary column: 15cm 3.0mm 3.5 μm SB-CN
Flow 0.05 ml/min from 5 min**

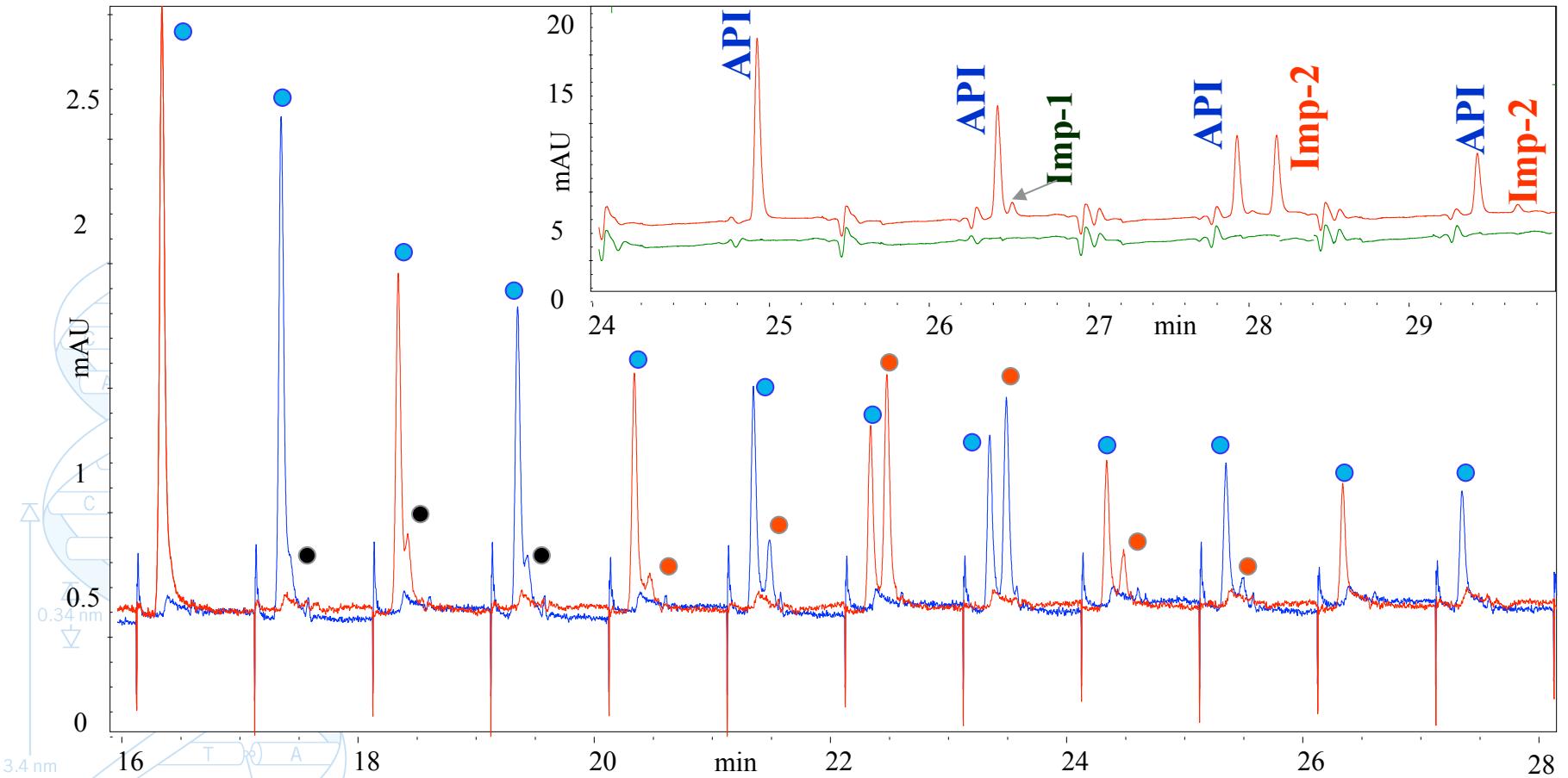
**Secondary column: Dual 5cm 4.6mm 1.8 μm SB-CN
Flow: 2.0 ml/min/column**

Secondary

Primary

Step 1: Modify primary column gradient elution to isocratic elution

Step 2: Slow primary column flow to enable for multiple sample transfer



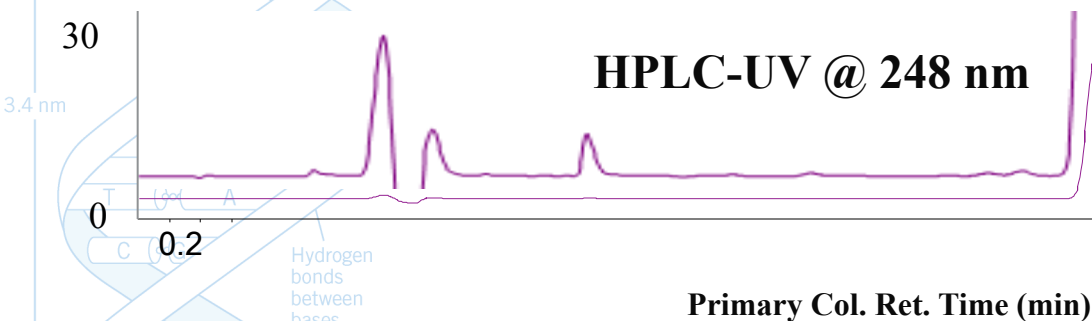
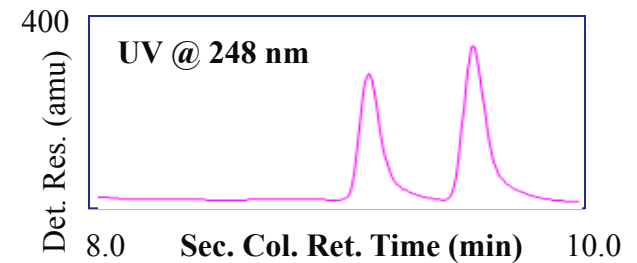
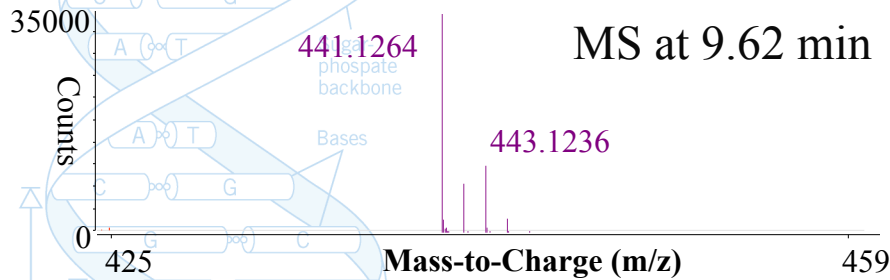
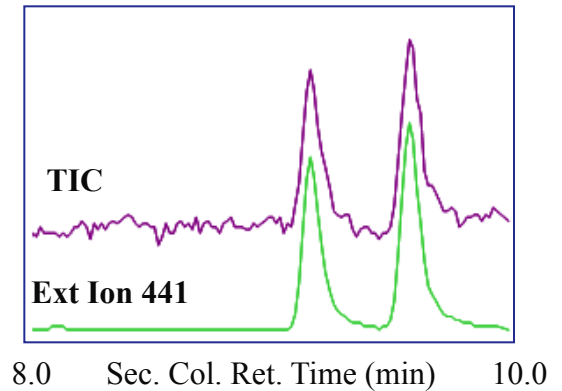
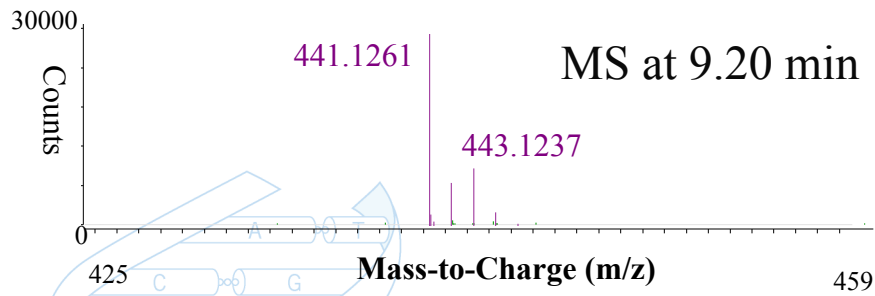
Minor impurity elutes in-between API and isomer ~ <0.02% (200 ppm)

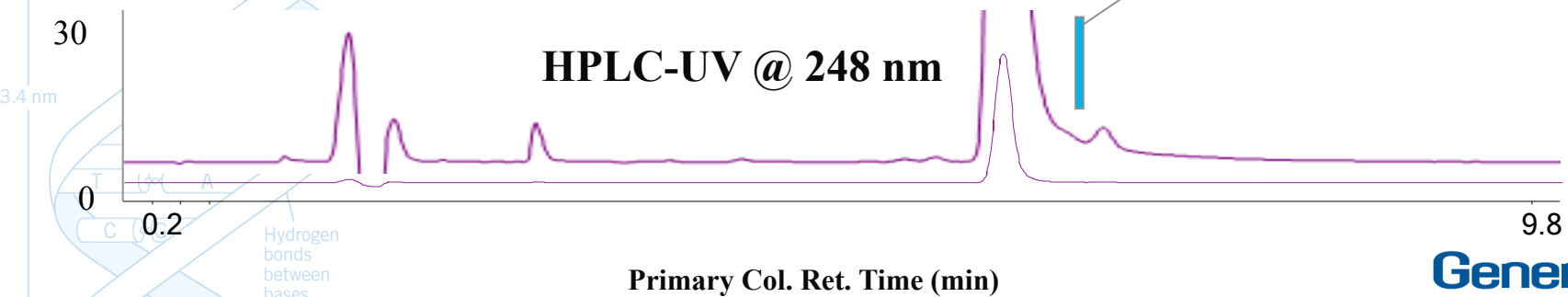
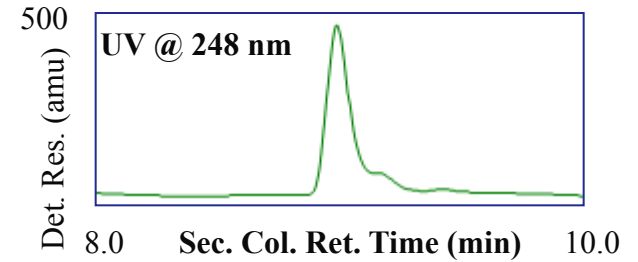
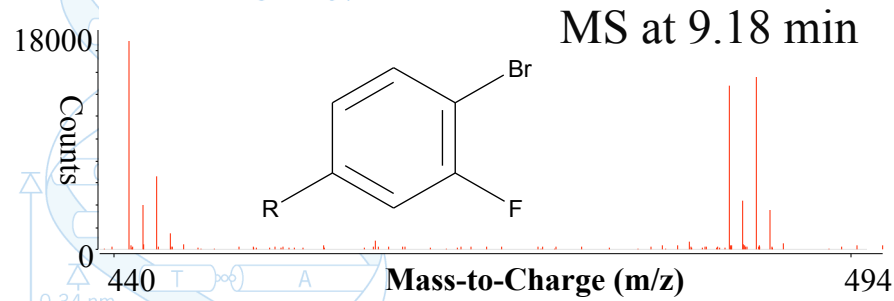
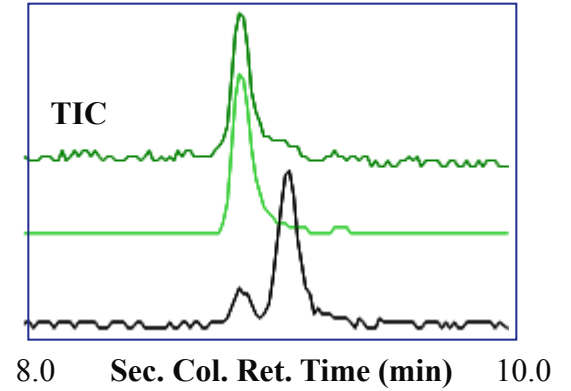
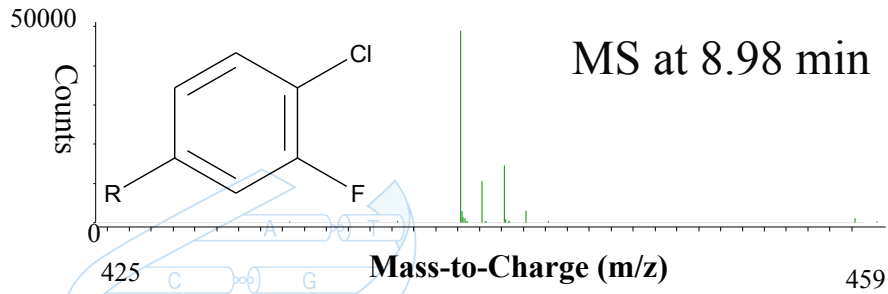
● API

● Impurity 1

● Impurity 2

Heart cutting LCxLC-MS (TOF) separation





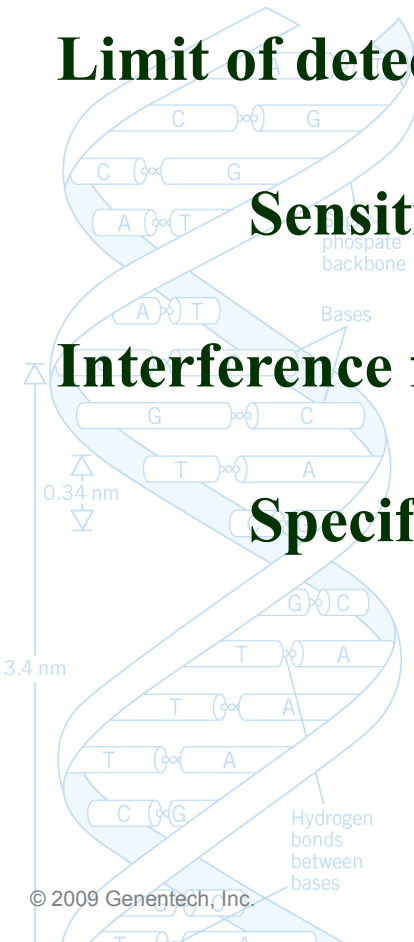
Challenges:

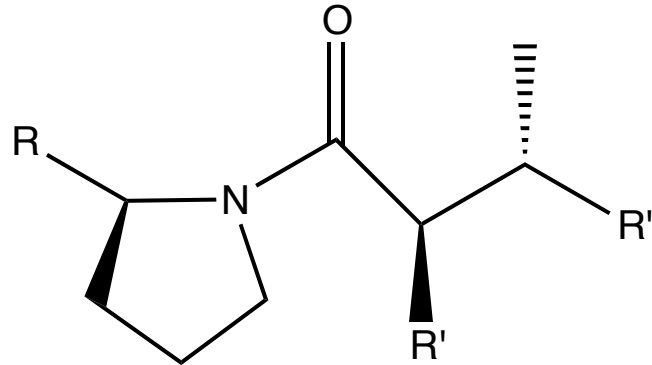
Limit of detection is usually in low part per million

Sensitive detection tool, usually MS

Interference from Sample matrix (conc. several mg/ml)

Specificity is often challenging

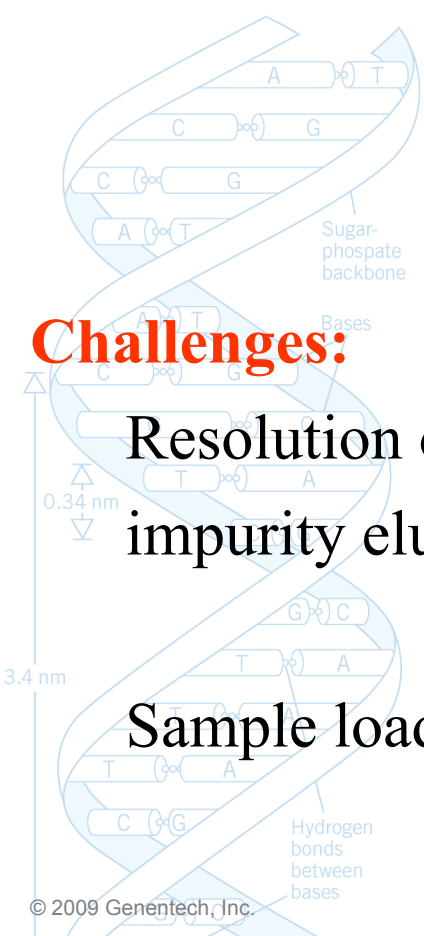


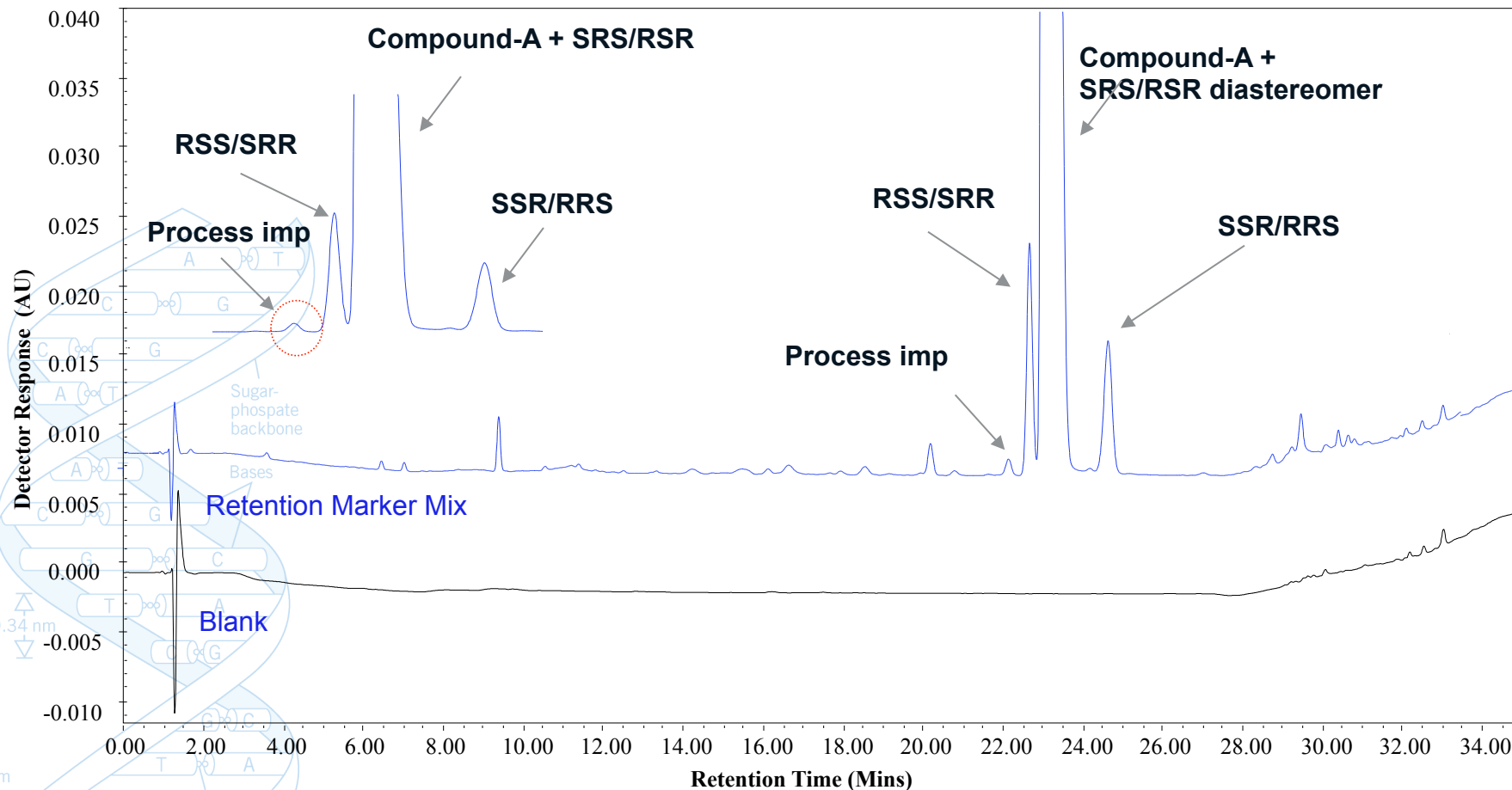


Challenges:

Resolution of three pairs of diastereomers plus a process related impurity eluting in the proximity of API

Sample loadability issue – resolution v/s sensitivity



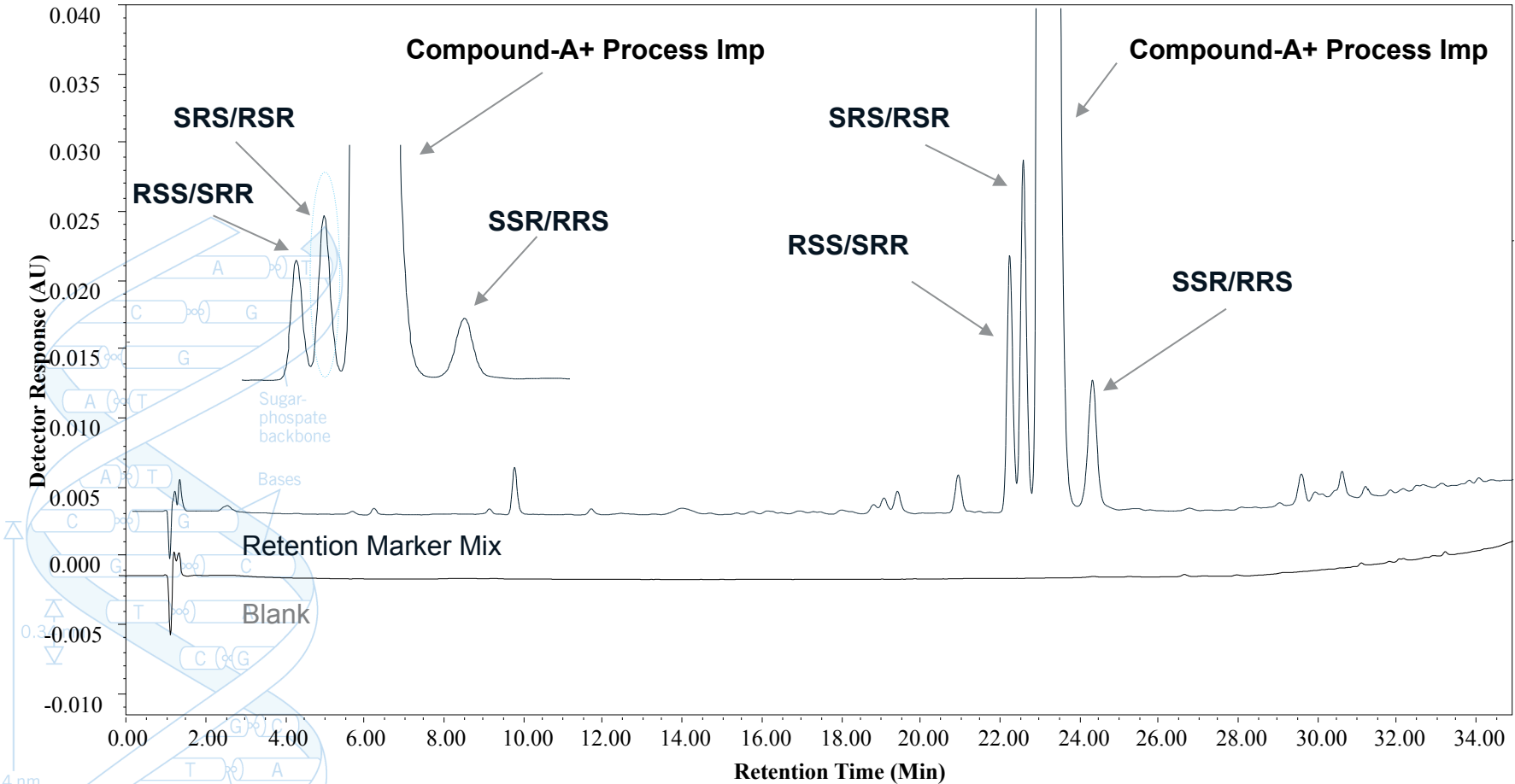


Column: Ace C18, 15 cm x 3.0mm, 3.0 micron / Detection 258 nm 0.8 ml/min

Gradient reversed phase separation – 20mM ammonium formate @ pH 3.7, 0.05% FA in acetonitrile gradient

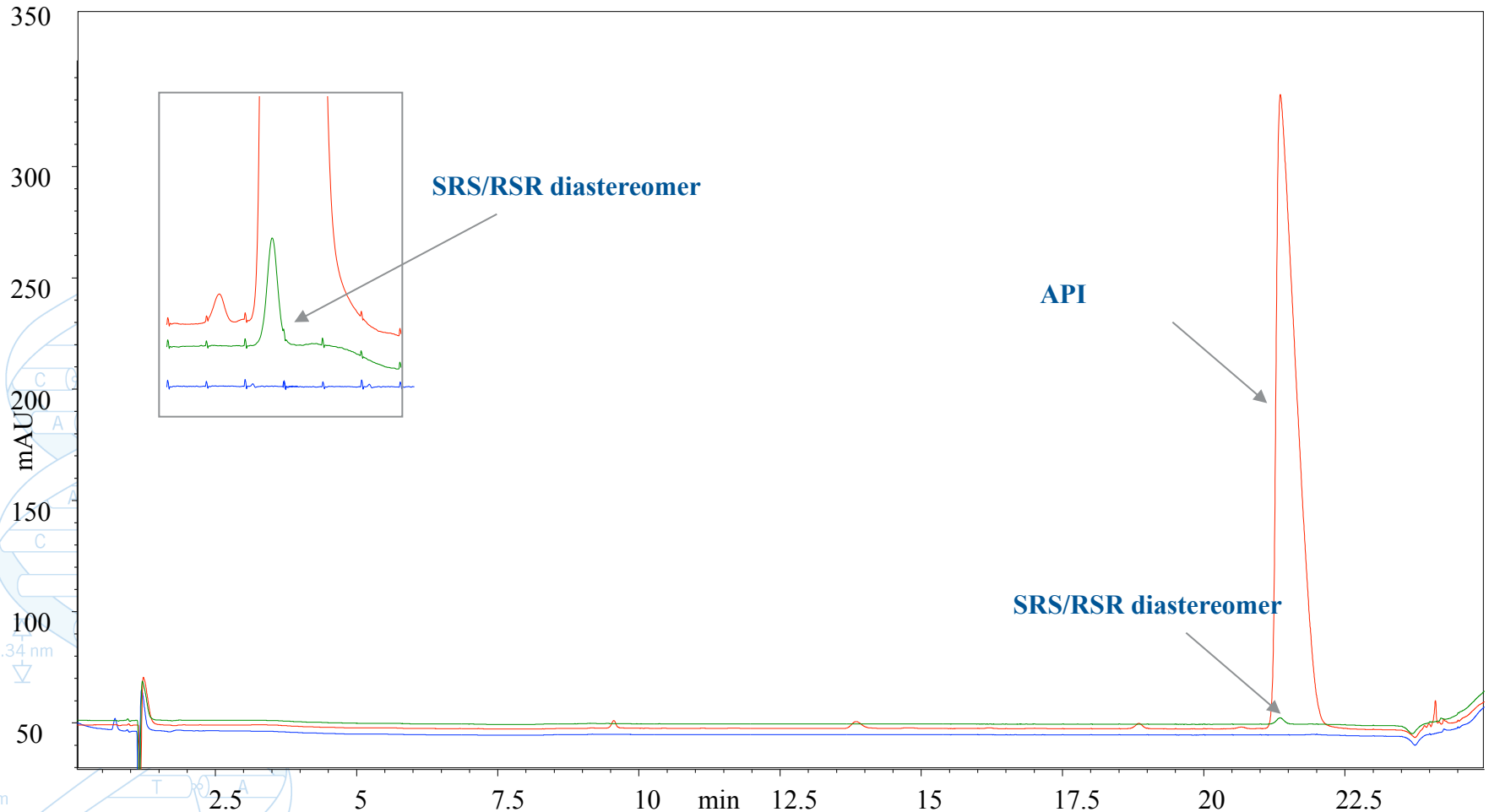
SRS/RSR diastereomers co-elutes with API **Genentech**

A Member of the Roche Group



Column: Water's XBridge Shield RP18, 15 cm x 3.0mm, 3.5 micron / Detection 258 nm, 0.6 ml/min
Gradient reversed phase separation – 20mM ammonium formate @ pH 3.7- Methanol gradient

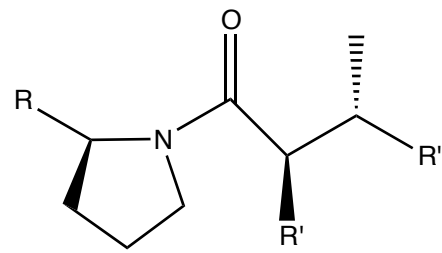
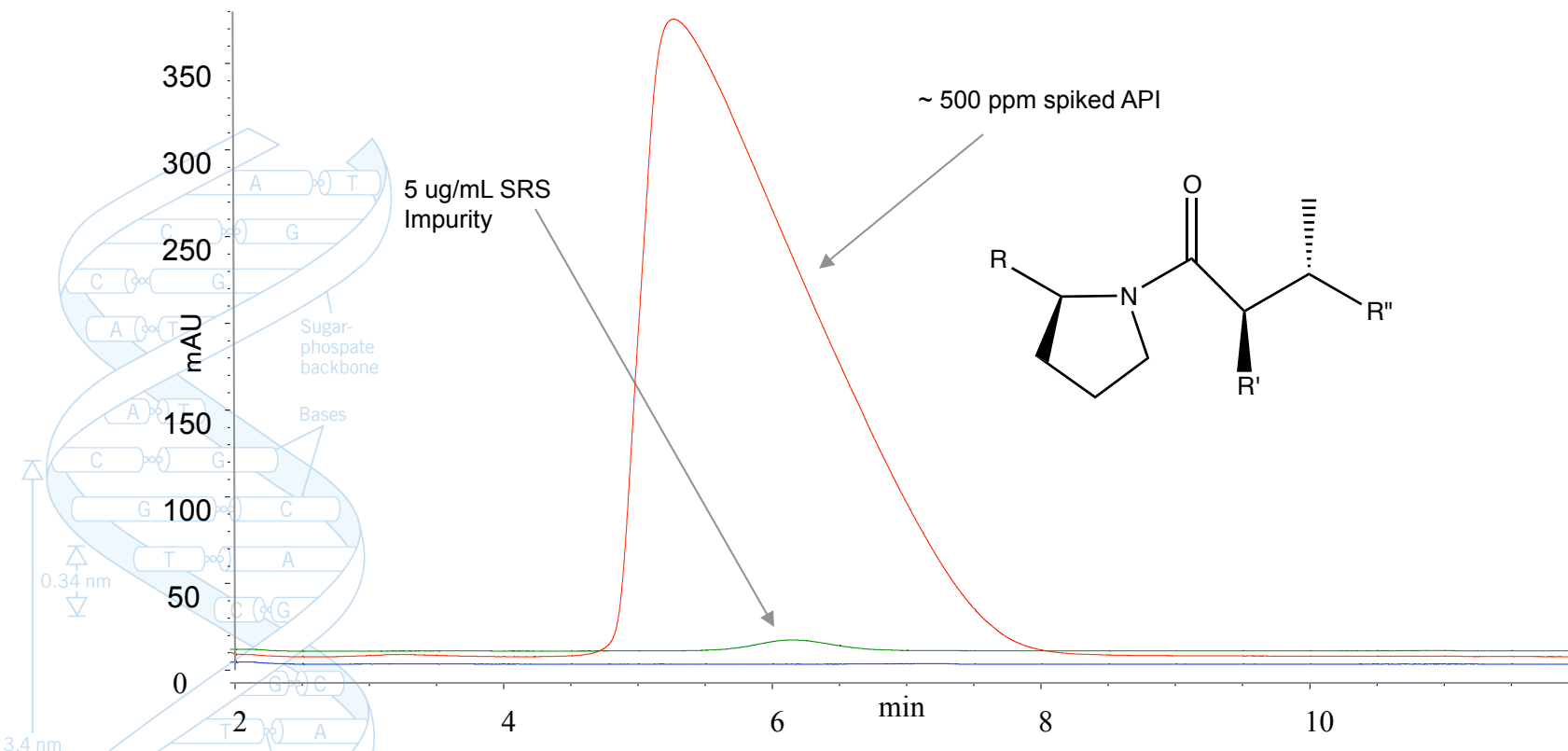
Process impurity co-elutes with API



Column: Ace C18, 15 cm 2.1 mm 3.0 micron

Gradient reversed phase separation – water – acetonitrile gradient with 0.2% H₃PO₄

SRS/RSR diastereomers co-elutes with the API

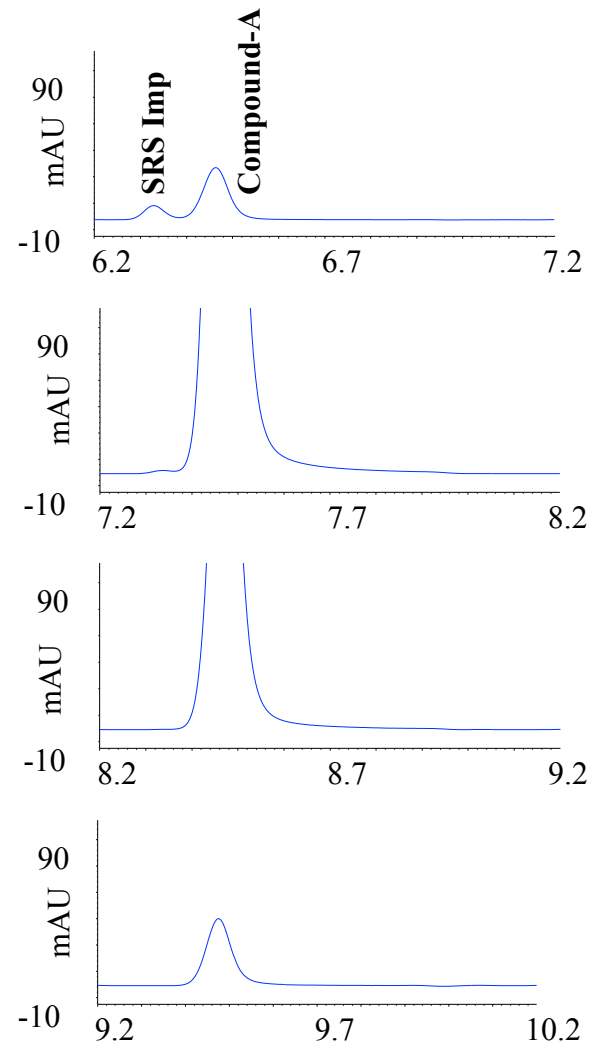
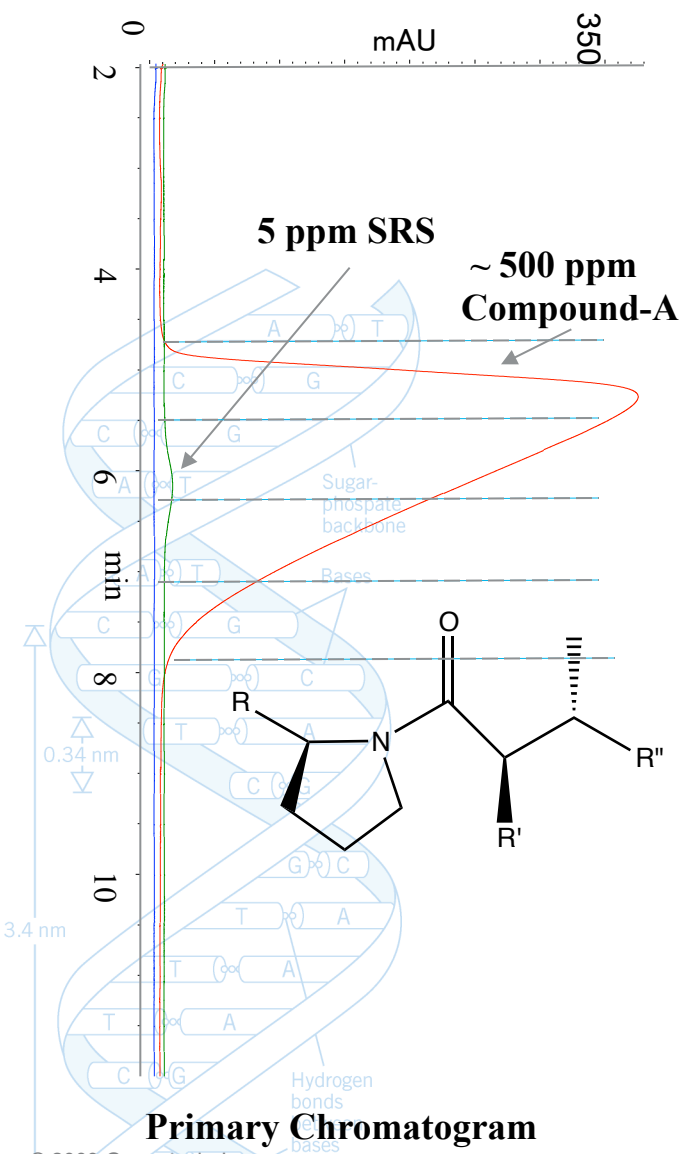


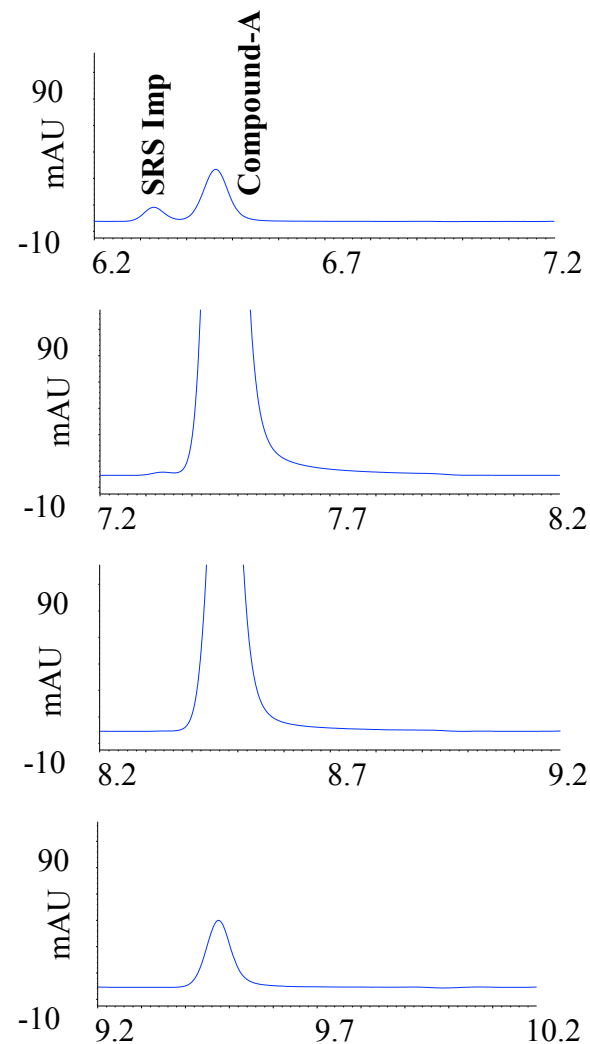
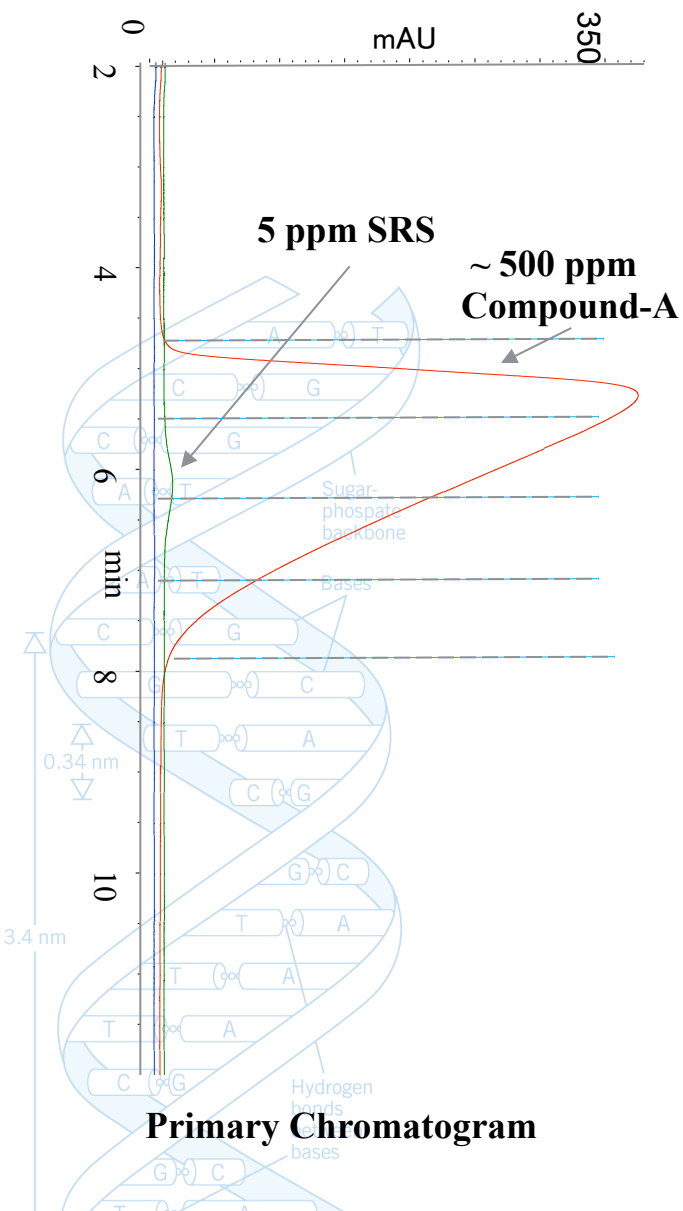
Column: Ace C18, 15 cm 2.1 mm 3.0 micron

Isocratic reversed phase separation – 70-30 water – acetonitrile with 0.2% H3PO4

Flow reduced from 0.8 ml/min to 0.1 ml/min at 2.0 – 10.0 minutes

SRS diastereomer co-elutes with API



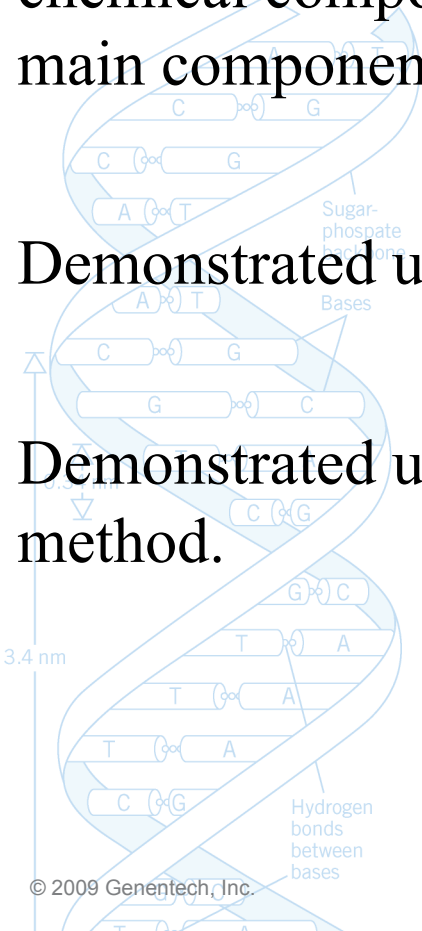


Conclusions

Successfully demonstrated resolving power of 2D-LC in resolving chemical components present in disproportionate levels in the midst of main component.

Demonstrated use of same phase for select 2D-LC separation.

Demonstrated use of 2D-LC separation to assess stability indicating method.



- James Girotti
- Ila Patel
- Nikhil Desai
- Michael Dong
- Kelly Zhang

* **Genentech**

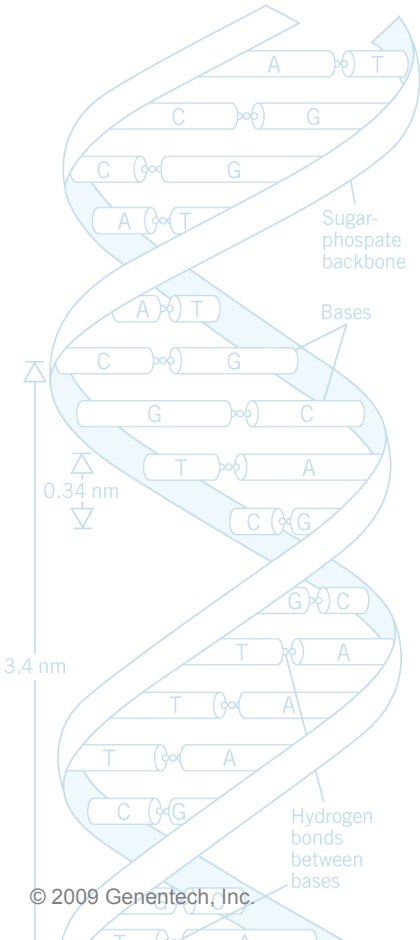
- Xin Linghu
- Stefan Koenig
- Scott Savage
- Greg Sowell
- Diane Carrera
- Brian Wong

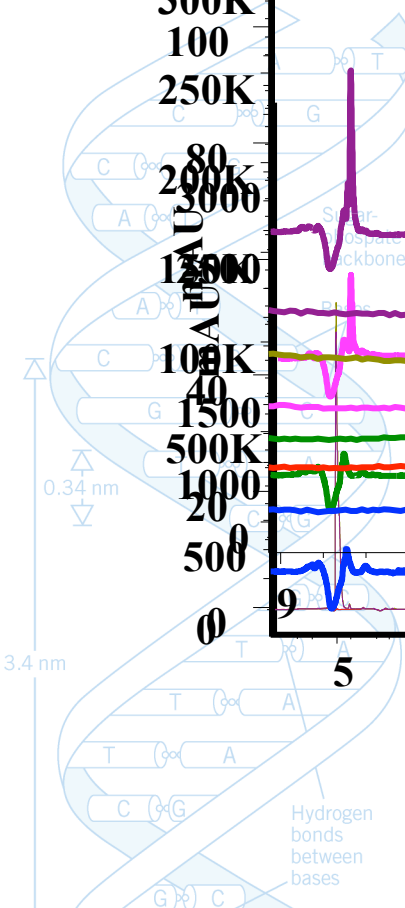
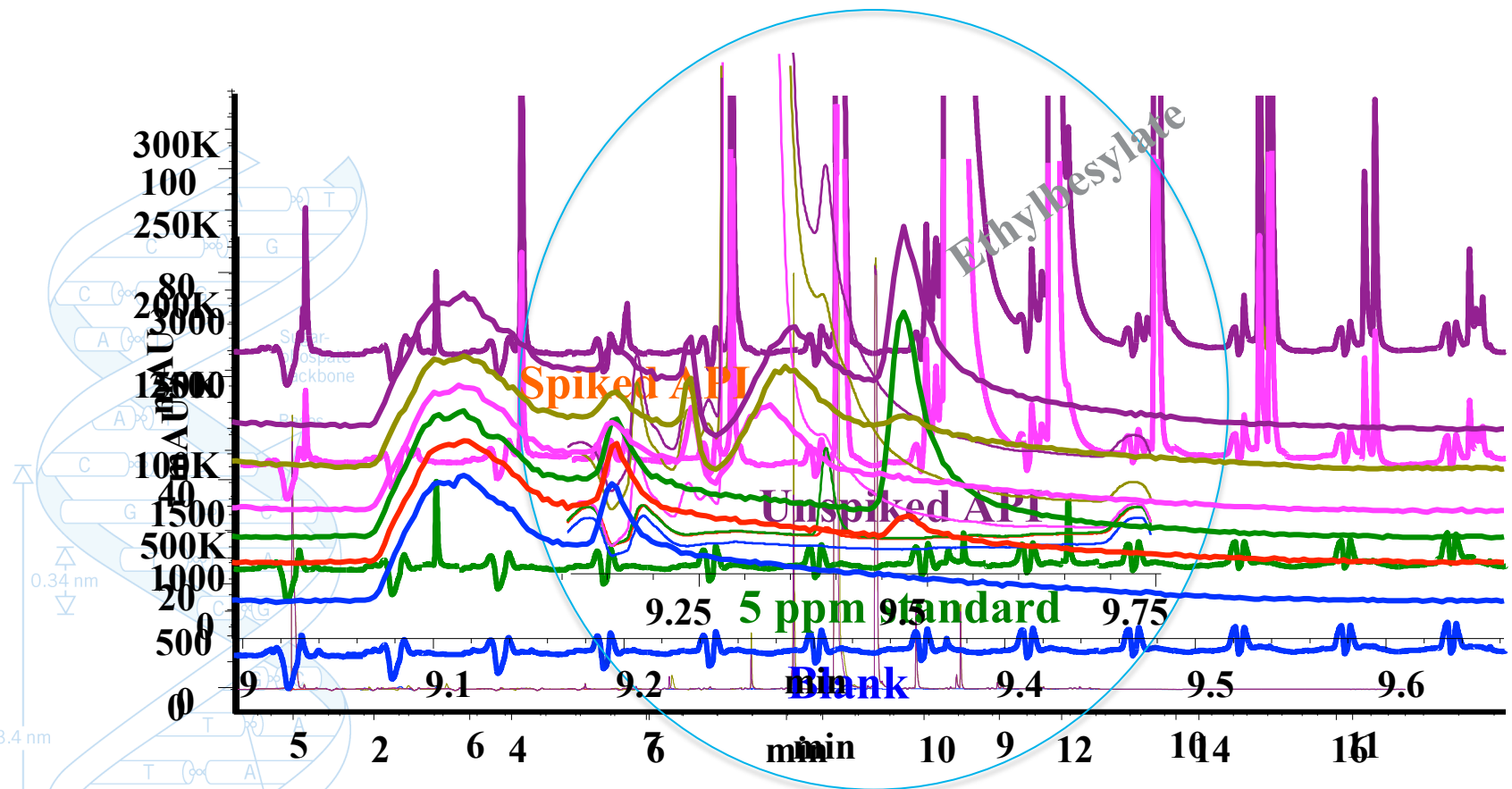
Michael Frank, Agilent Technologies

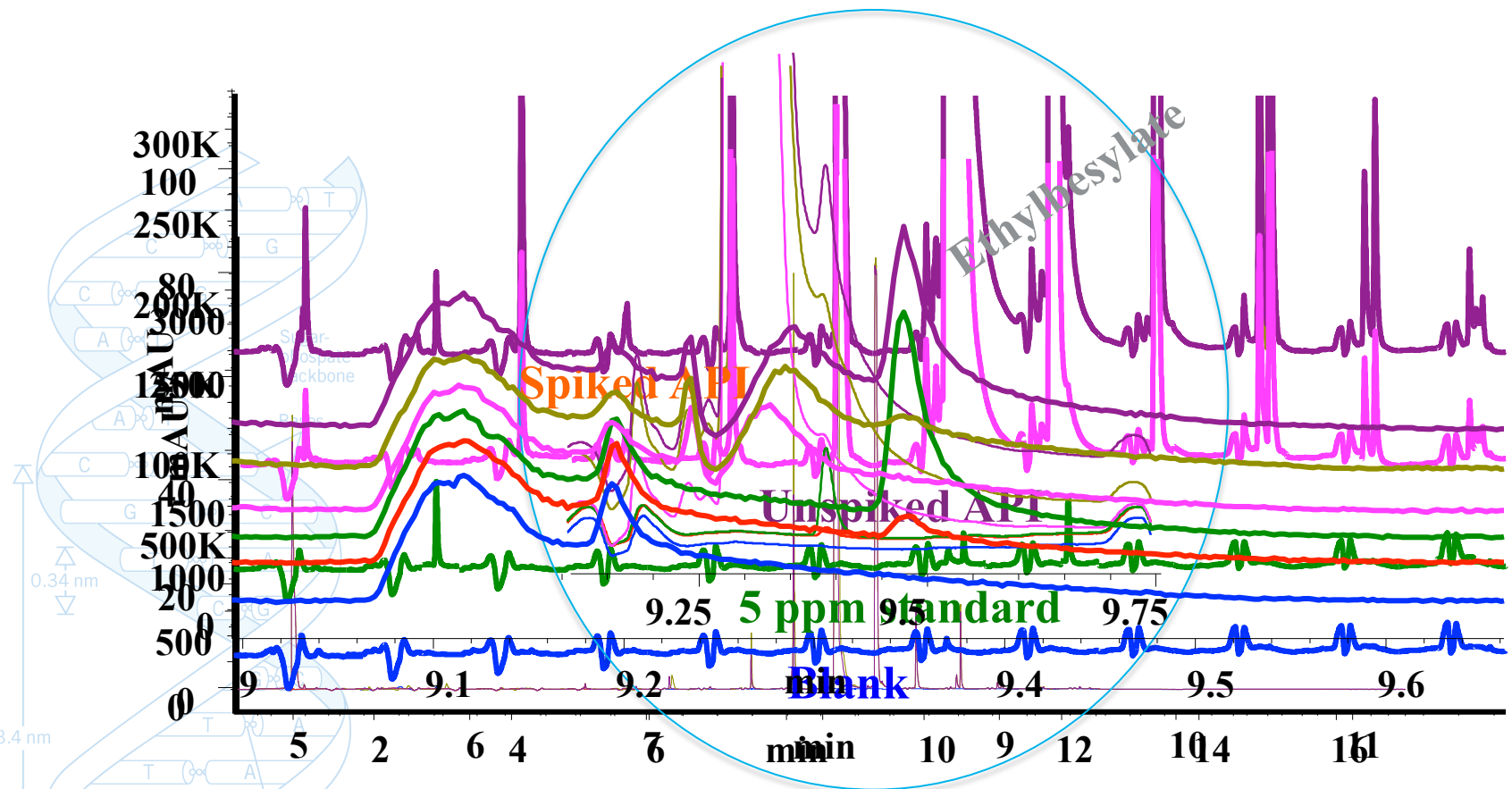
Lisa Zang, Agilent Technologies

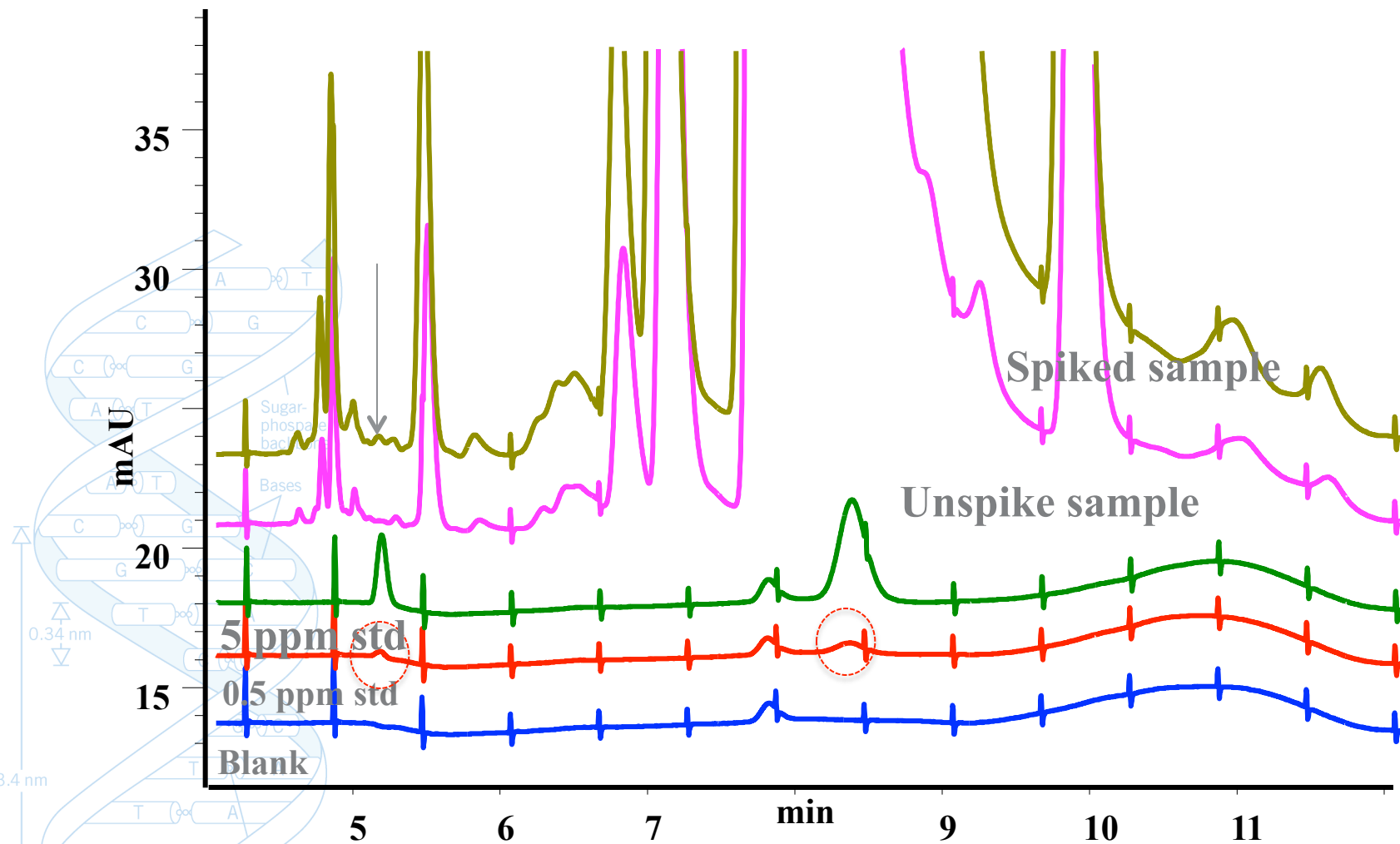


Thanks









Ist Dim: Zorbax Phenylhexyl: 5 cm x 2.1 mm x 1.8 micron

Modulation: Once a minute; Gradient elution