# RPLC/HILIC/API-MS: polarity extended analysis for organic molecules in water bodies

**Sylvia Grosse and Thomas Letzel**

Analytical Research Group

Chair of Urban Water Systems Engineering

Technical University of Munich

**Ixia, Rhodes, 1st September, 2015**

# content

- polarity extension RPLC with HILIC
- HILIC retention mechanisms:
  - adsorption
  - distribution (water layer)
  - electrostatic interaction
- stationary phases and mobile phases in HILIC
- serial HILIC-RP-MS coupling
- application
- conclusion

# Why polarity extension?
# Polar and nonpolar molecules

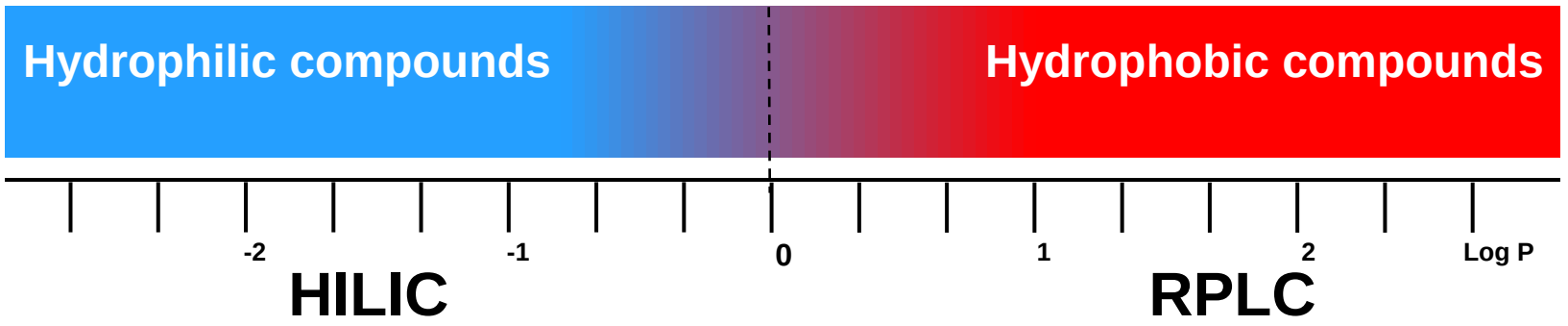
Partition coefficient (P): $\log P = \log ([solute]_{oct}/[solute]_{wat})$

Distribution coefficient (D): $\log D = \log ([solute]_{oct}/([solute]_{wat}^{ionized} + [solute]_{wat}^{neutral})$

*(For charged molecules)*

**Log P < 0**
**or**
**Log D < 0**

**Log P > 0**
**or**
**Log D > 0**



# Separation of polar and nonpolar compounds

**Hydrophilic analytes**

**Hydrophobic analytes**

**HILIC**

**hydrophylic interaction liquid chromatography**

**RPLC**

**Reversed phase liquid chromatography**

NP stationary phases

RP eluents

**Typical mobile phase**

**ACN/$H_2O$**

**$H_2O$/ACN**

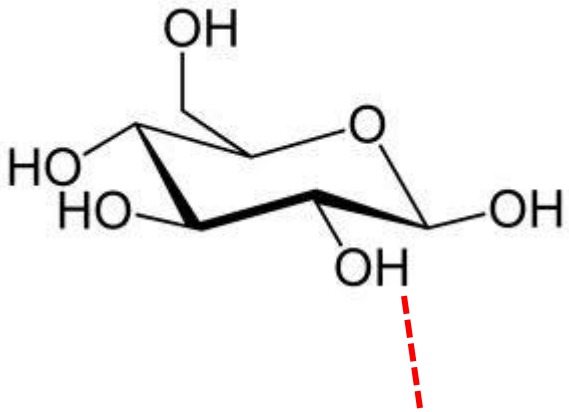
**RPLC *vs.* HILIC:**

- Orthogonal
- Use of the same solvents
- MS compatible

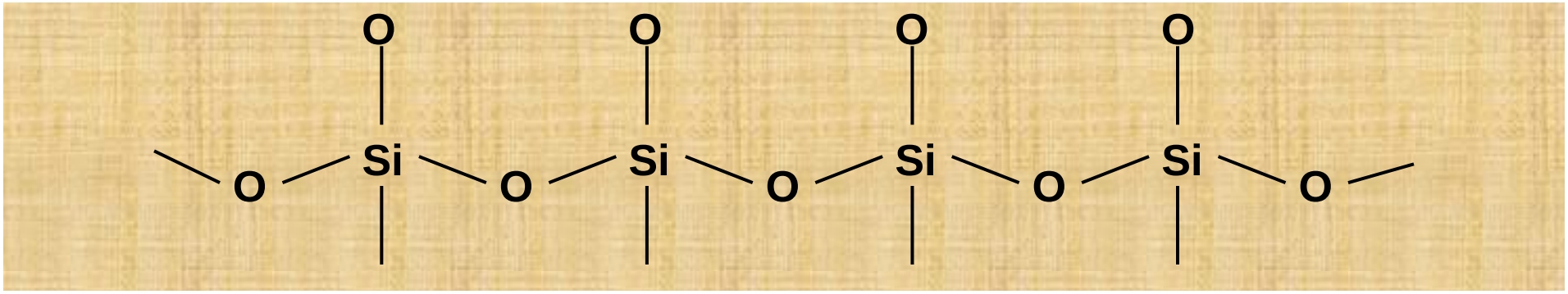
# Retention mechanisms

ADSORPTION

**Analyte - Stationary Phase**

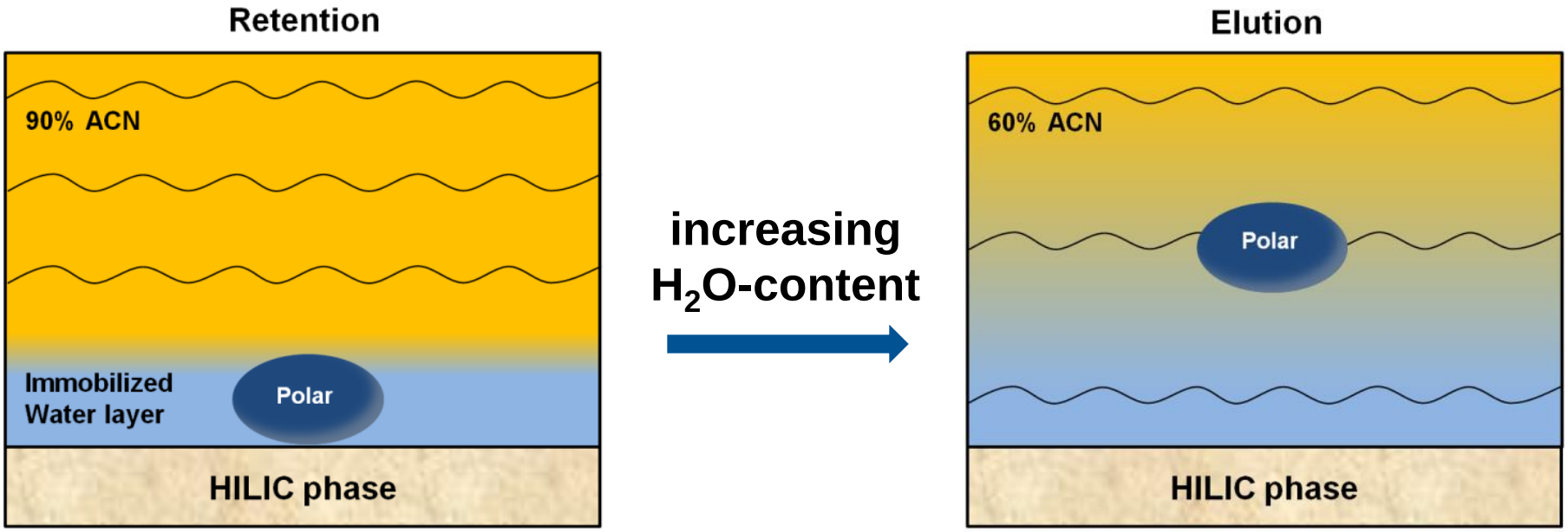



- ✓ **Hydrogen bonding**
- ✓ **Dipole - Dipole**



# Retention mechanisms

DISTRIBUTION



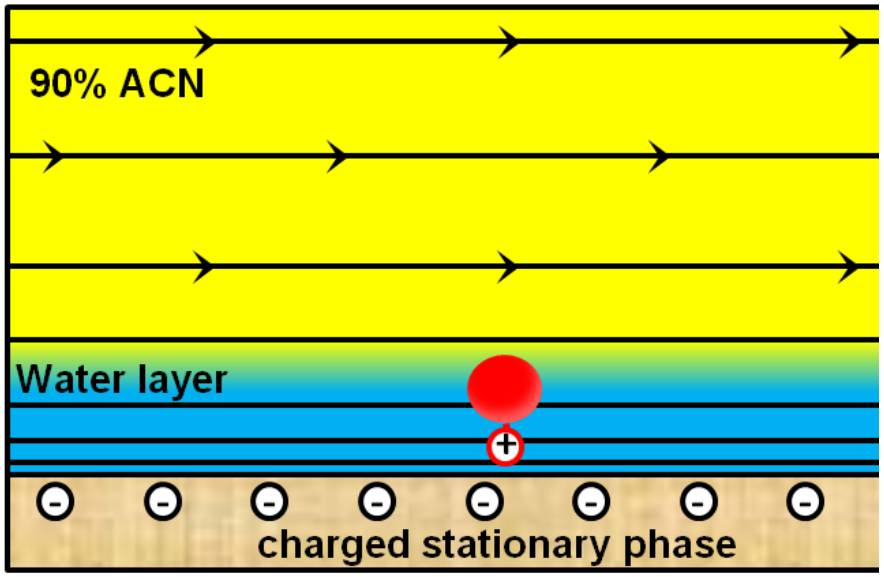

✓ **at least 2-3% water are essential in the mobile phase!**

Greco et al., J. Chrom. Sci, 2013

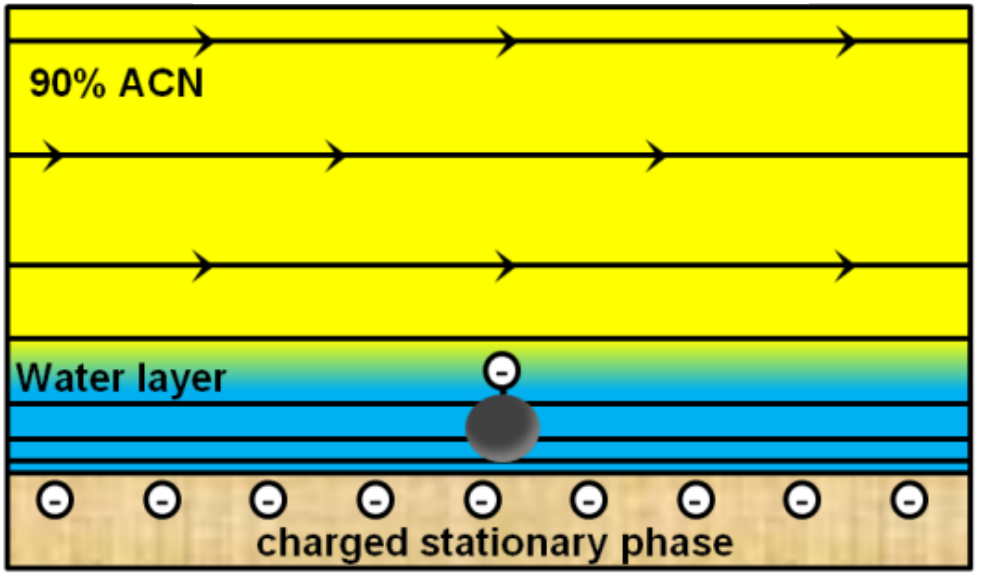
# Retention mechanisms

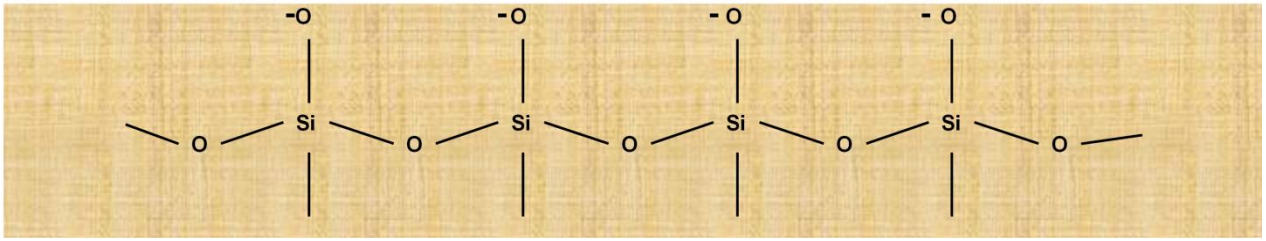
ELECTROSTATIC INTERACTION

## Electrostatic attraction

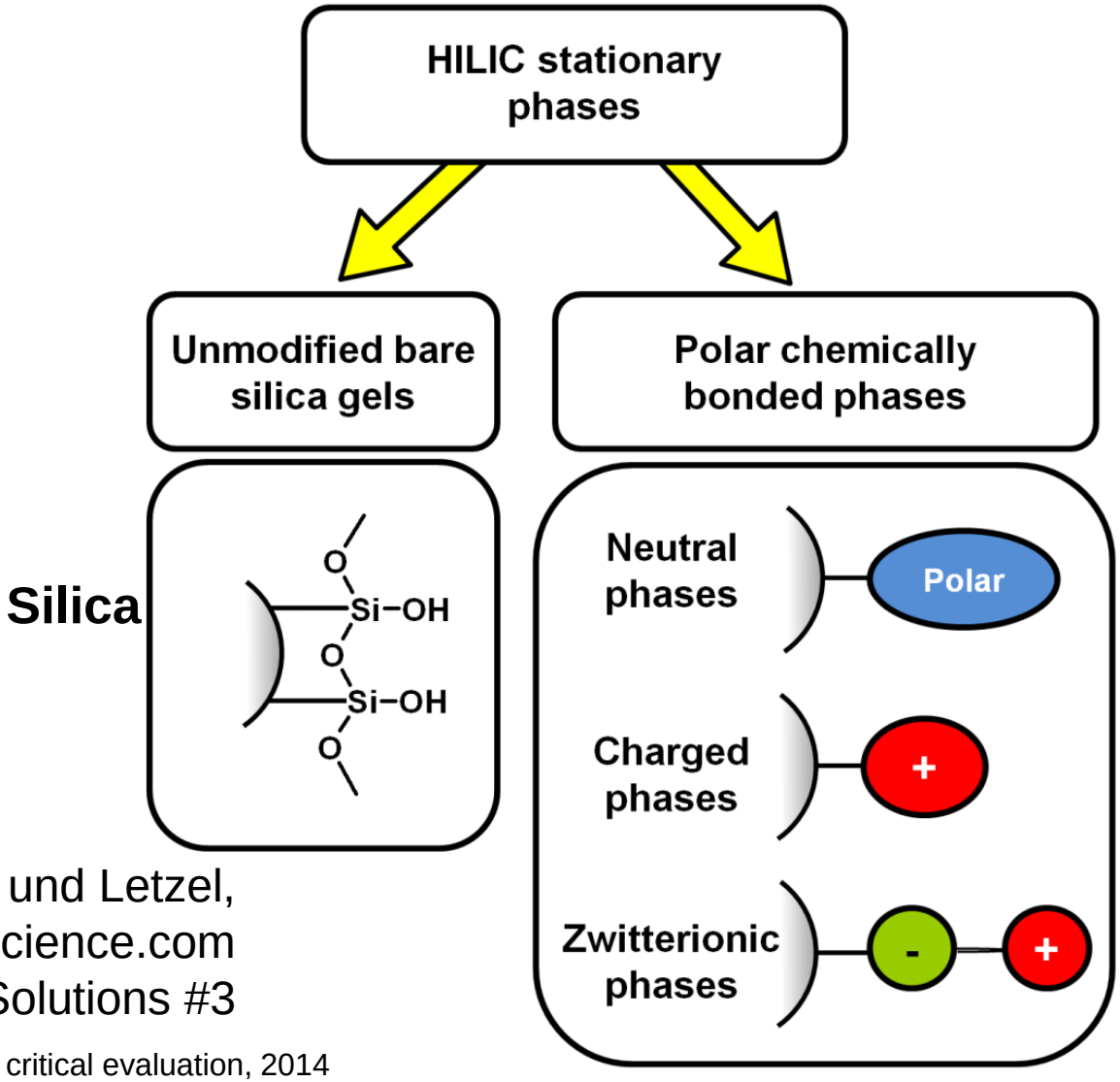



## Electrostatic repulsion



# Stationary Phases




**Diol, Amide, Cross-linked diol**

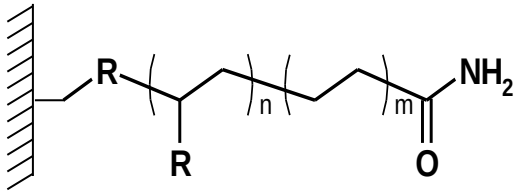
**Aminopropyl**

**Sulfobetaine**

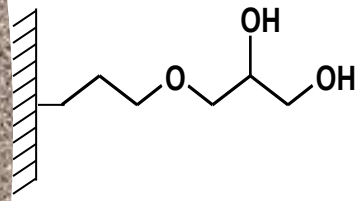
Greco und Letzel, www.sepscience.com HILIC Solutions #3

Guillarme, HILIC: a critical evaluation, 2014

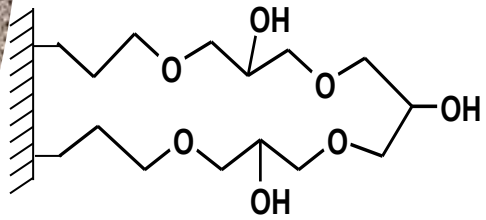
# Stationary Phase: neutral




**Amide**




**Diol**

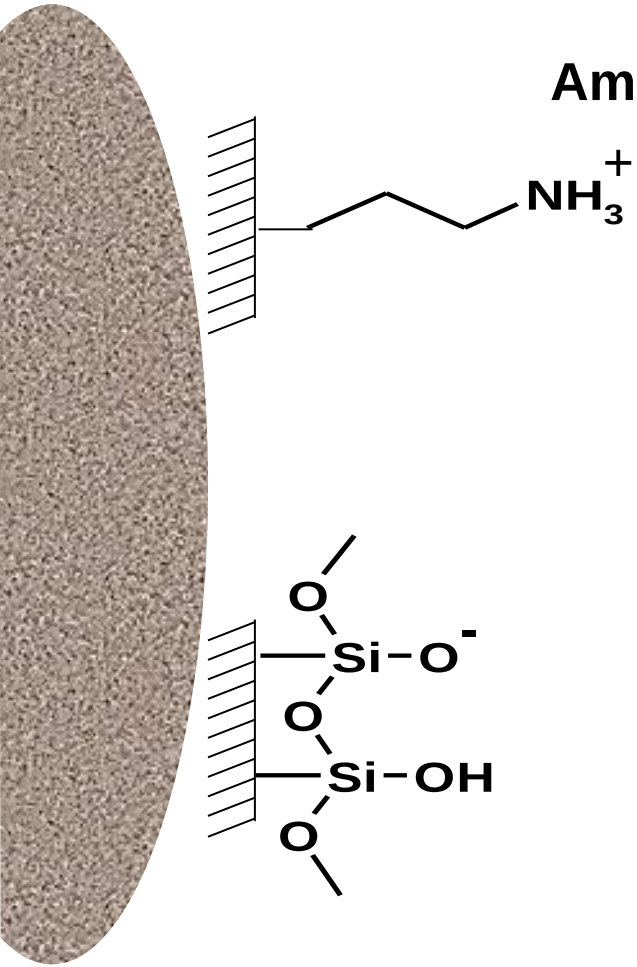



**Cross-linked Diol**

| Distribution | Electrostatic interaction | Adsorption |
|---|---|---|
| ✓ | ✗ | ✓ |
| ✓ | ✗ | ✓ |
| ✓ | ✗ | ✓ |

Greco et al., J. Chrom. Sci, 2013

# Stationary Phase: ionic

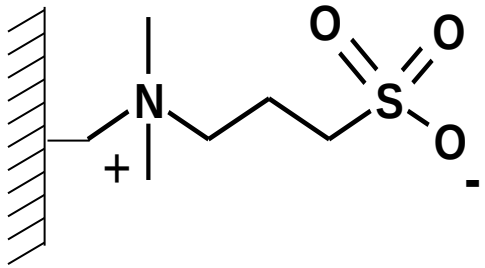


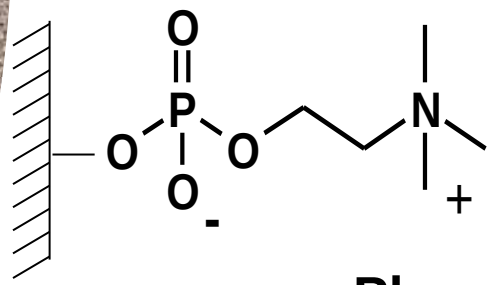

| Distribution | Electrostatic interaction | Adsorption |
|---|---|---|
| ✓ | ✓ Anion exchange | ✓ |
| ✓ | ✗ pH < 4/5<br>✓ pH > 4/5<br>Cation exchange | ✓ |

Greco et al., J. Chrom. Sci, 2013

# Stationary Phase: zwitterionic

**Sulfobetaine (ZIC-HILIC)**



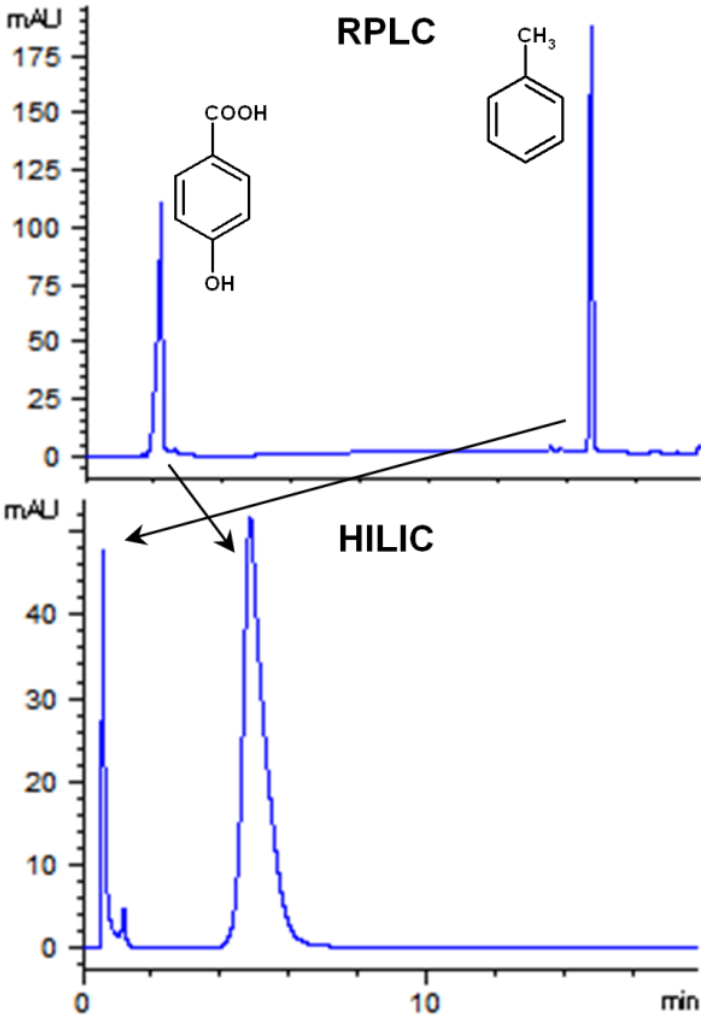




**Phosphorylcholine (ZIC-cHILIC)**

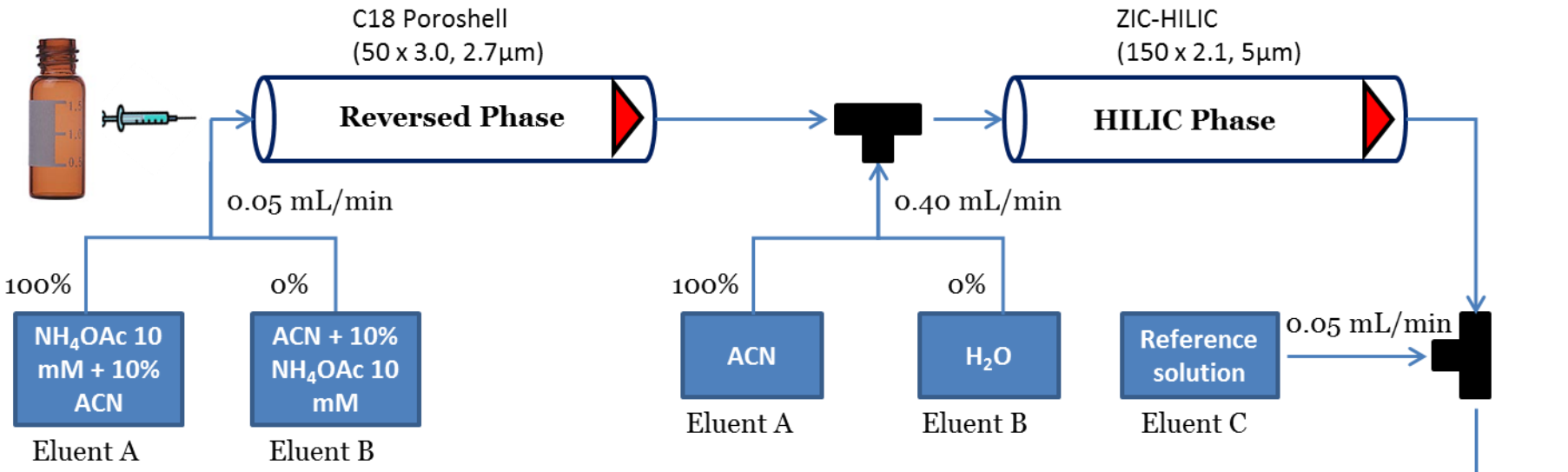
| Distribution | Electrostatic interaction | Adsorption |
|---|---|---|
| ✓ | ✓ | ✓ |
| ✓ | ✓ weak | ✓ |

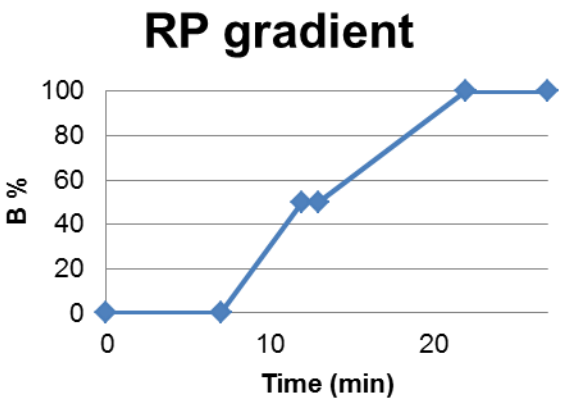
Greco et al., J. Chrom. Sci, 2013

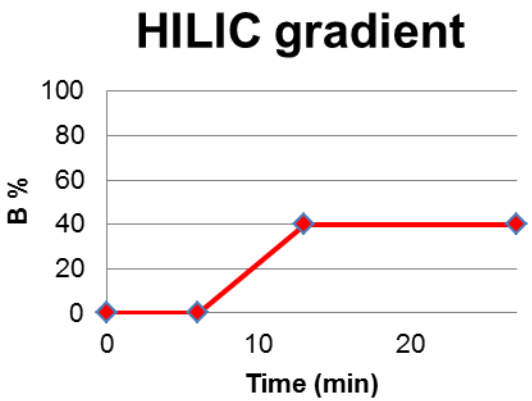
# Orthogonality RPLC and HILIC



# RPLC-HILIC coupling

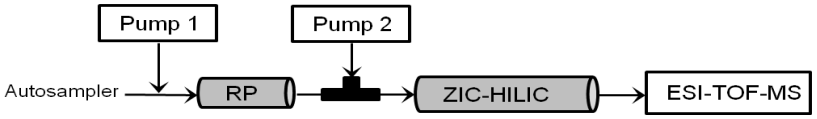


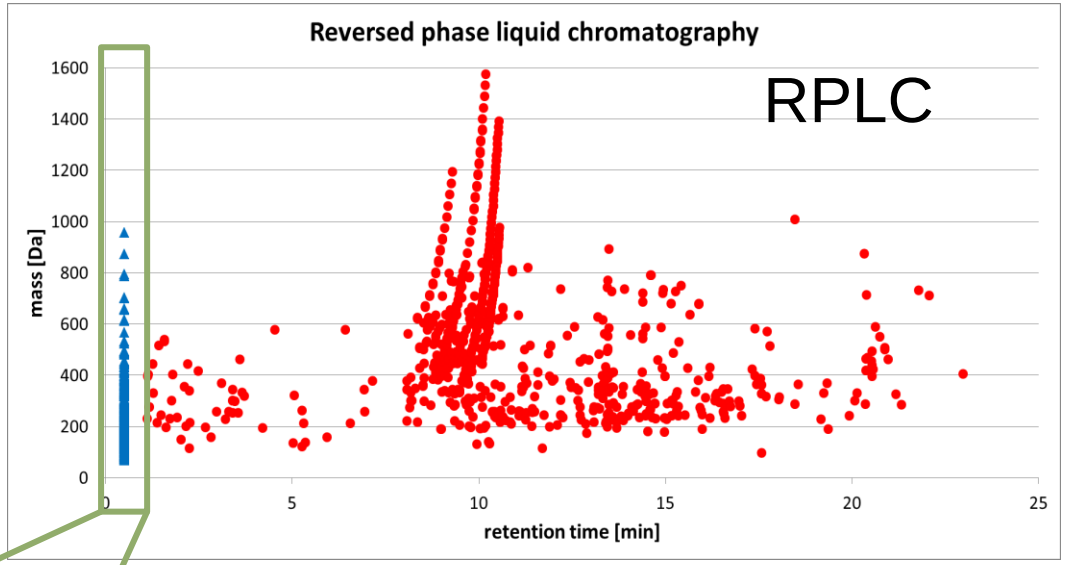



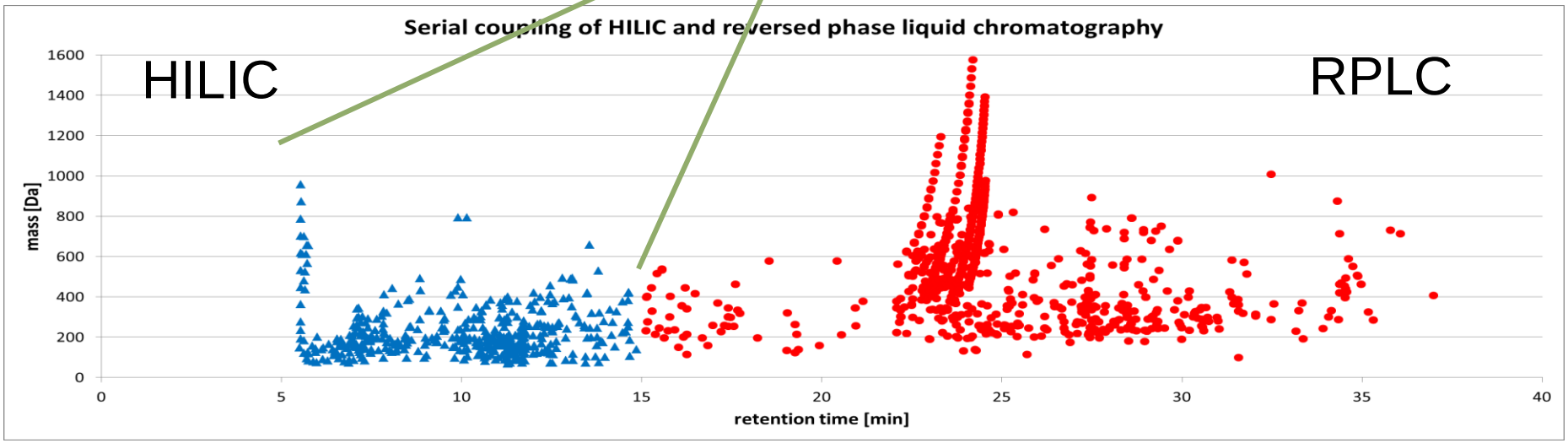



# Polarity Extension

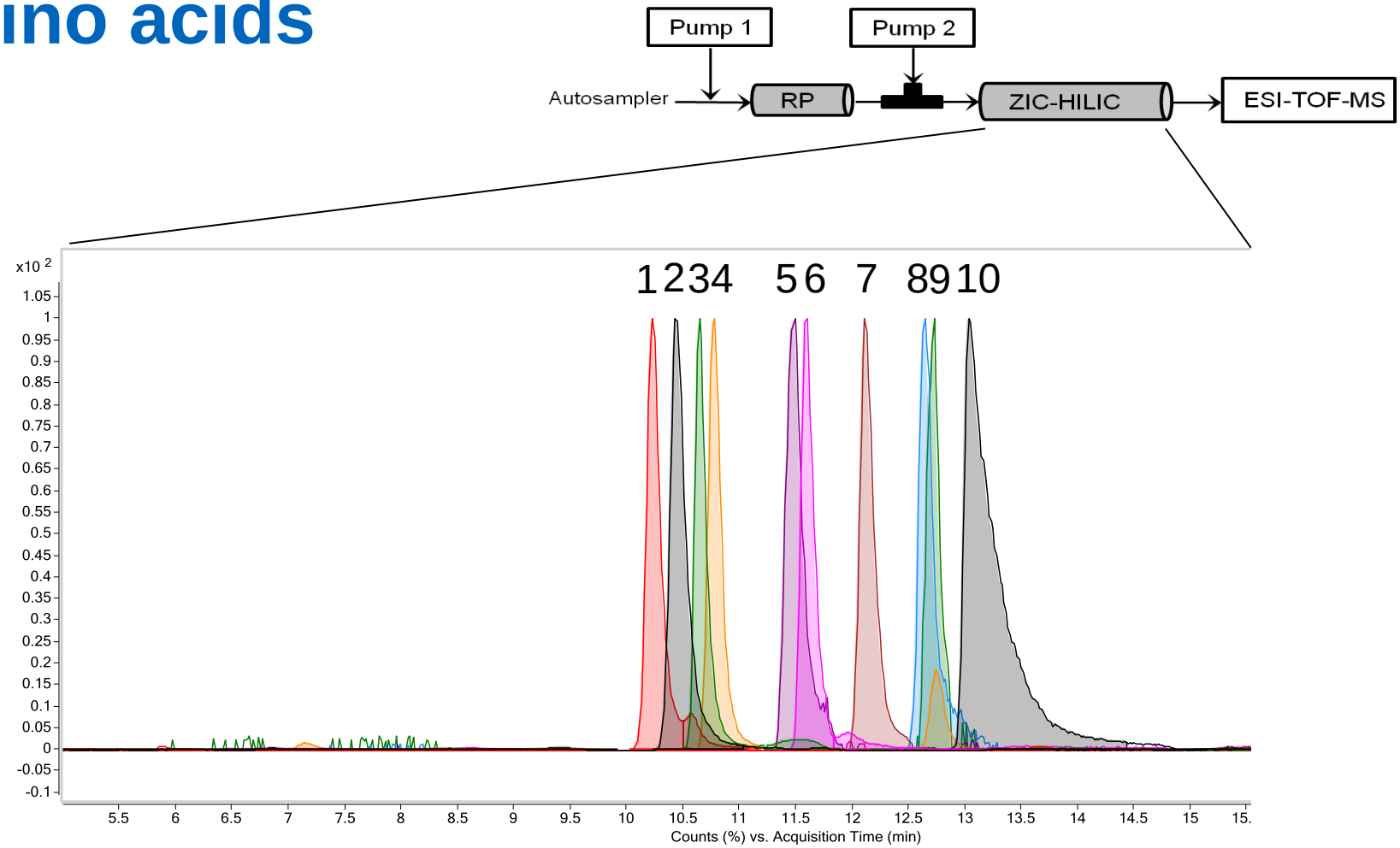
No HILIC



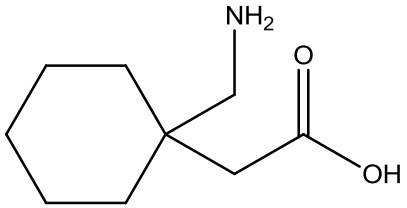



# HILIC-RPLC application I: Amino acids

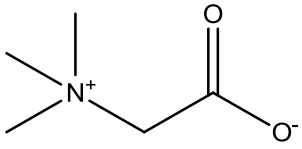



1 Phe; 2 Leu; 3 Trp; 4 Ile; 5 Val; 6 Tyr; 7 Pro; 8 Thr; 9 Ala; 10 Asn

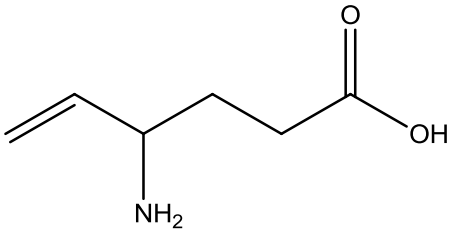
# HILIC-RPLC application II: pharmaceuticals and neurotransmitter



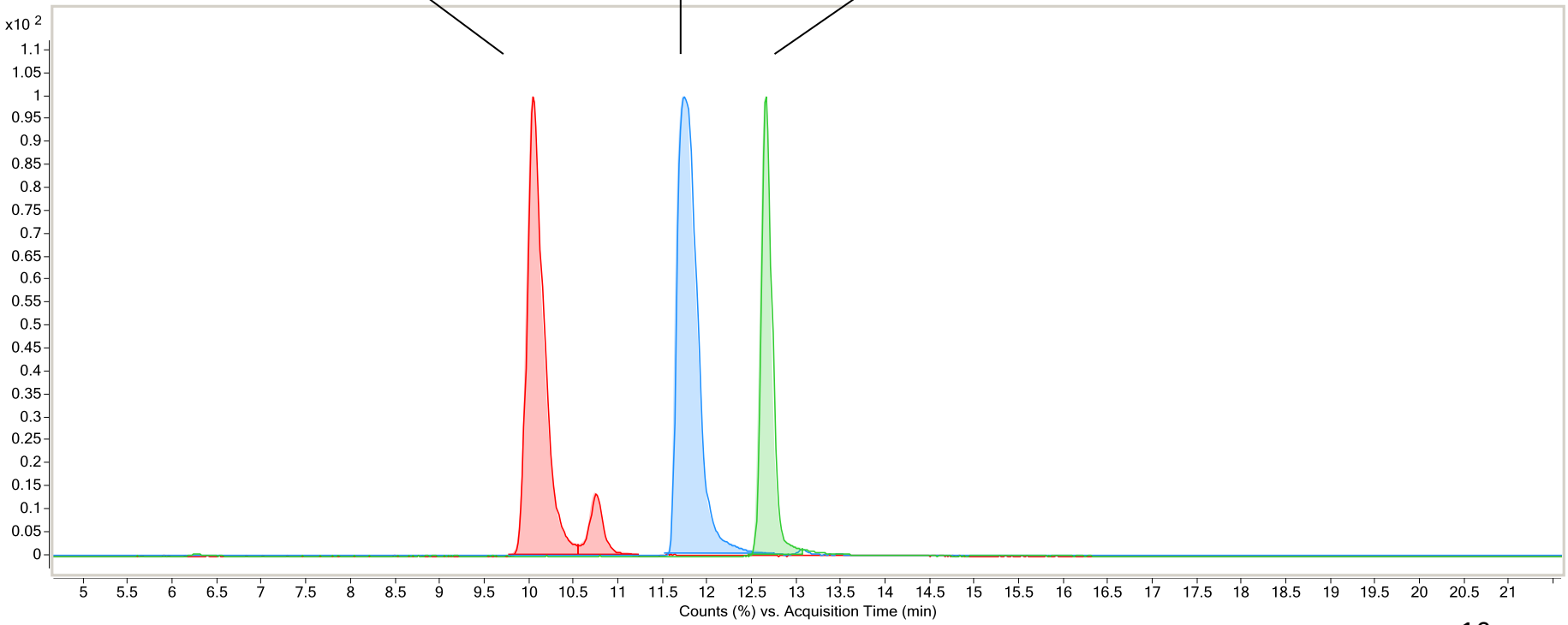

Gabapentin




Betaine




Vigabatrin



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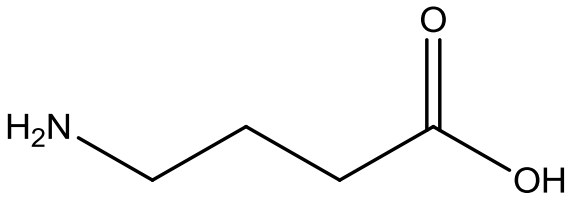
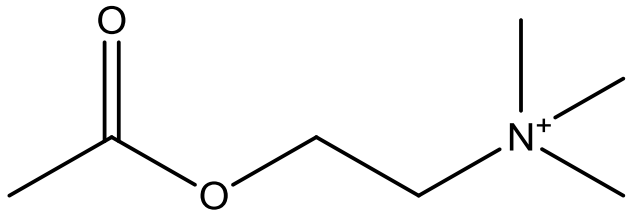
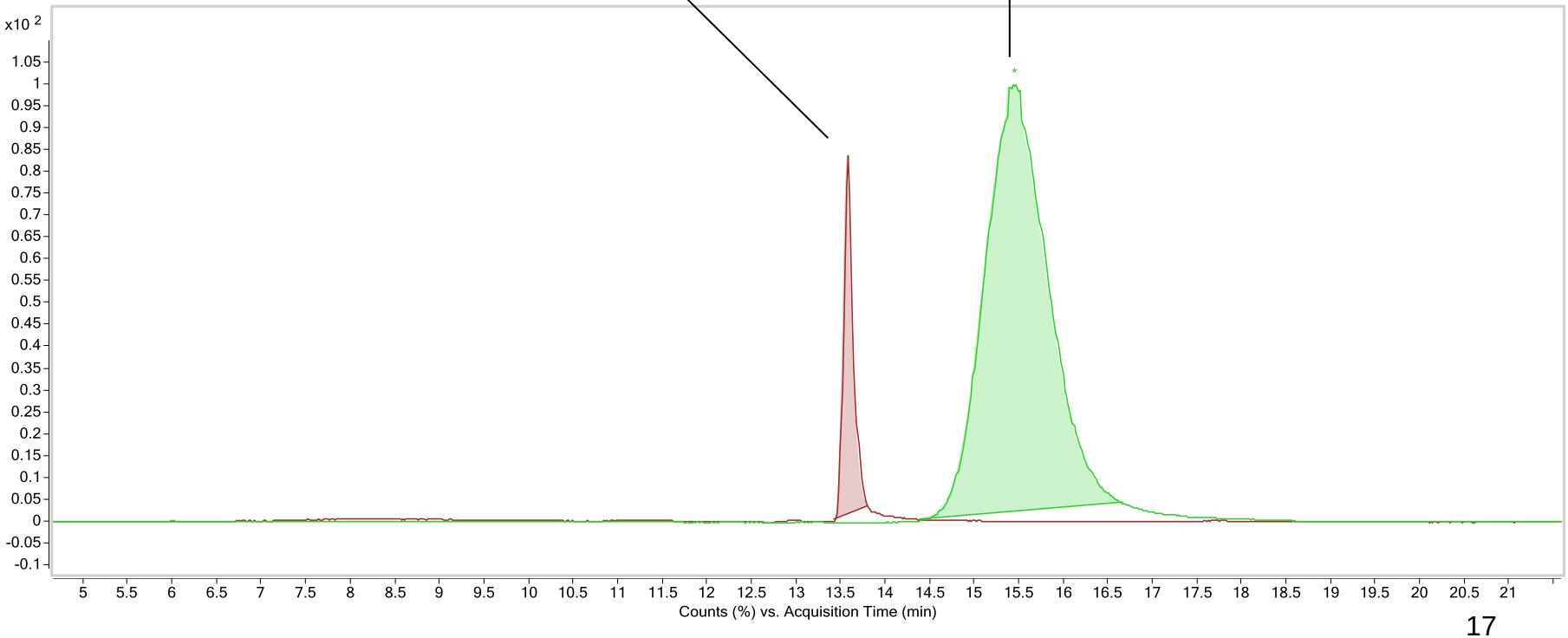


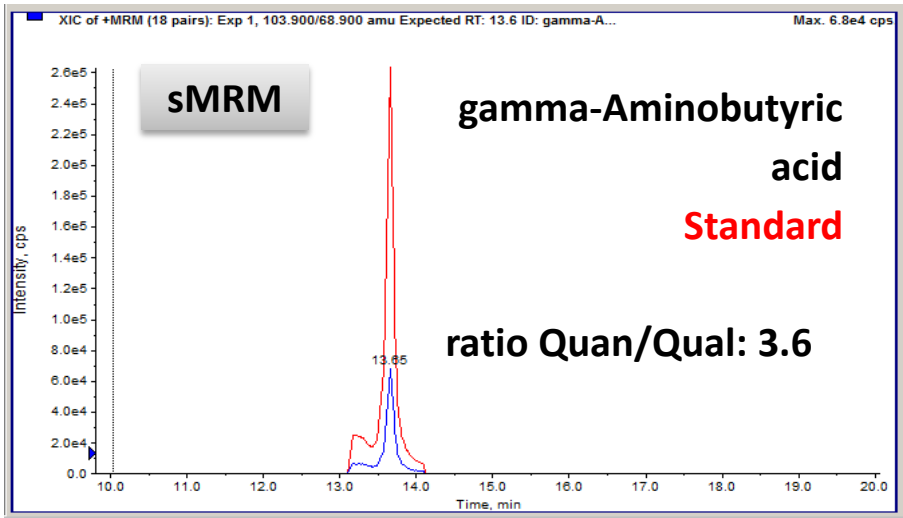

gamma aminobutyric acid?

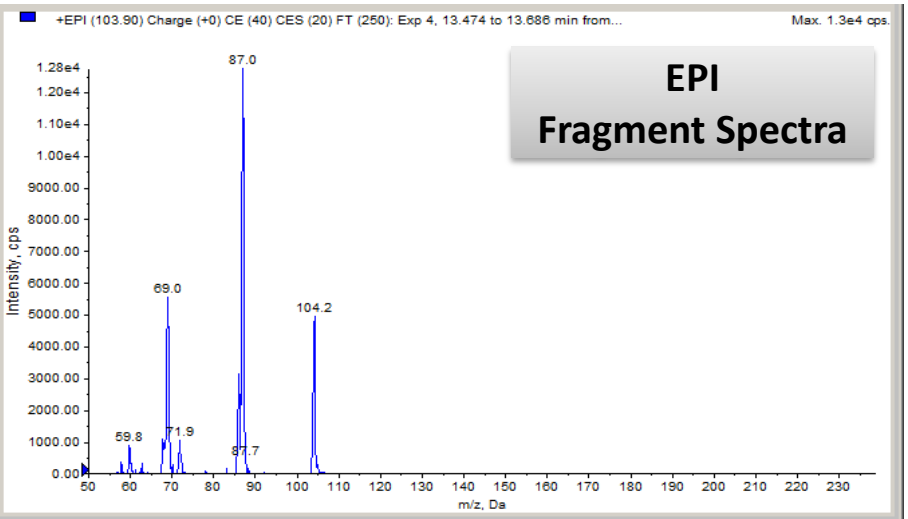


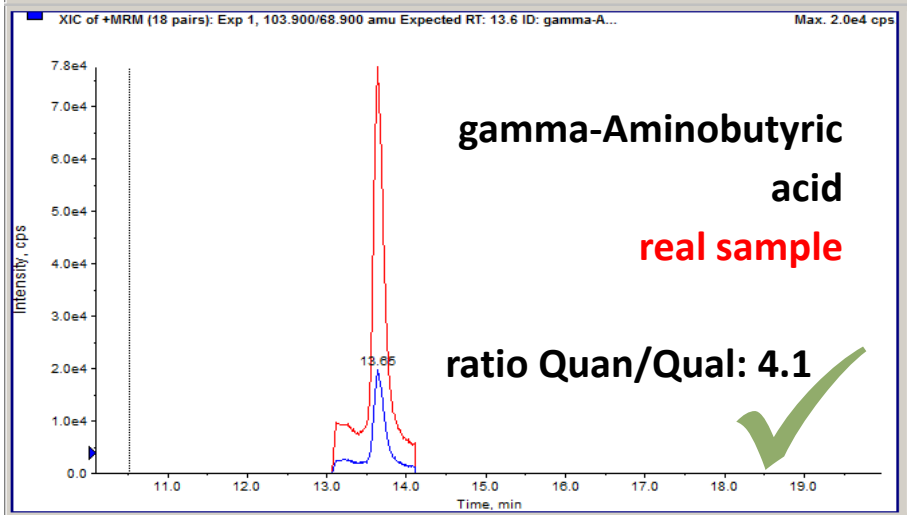

Acetylcholine?

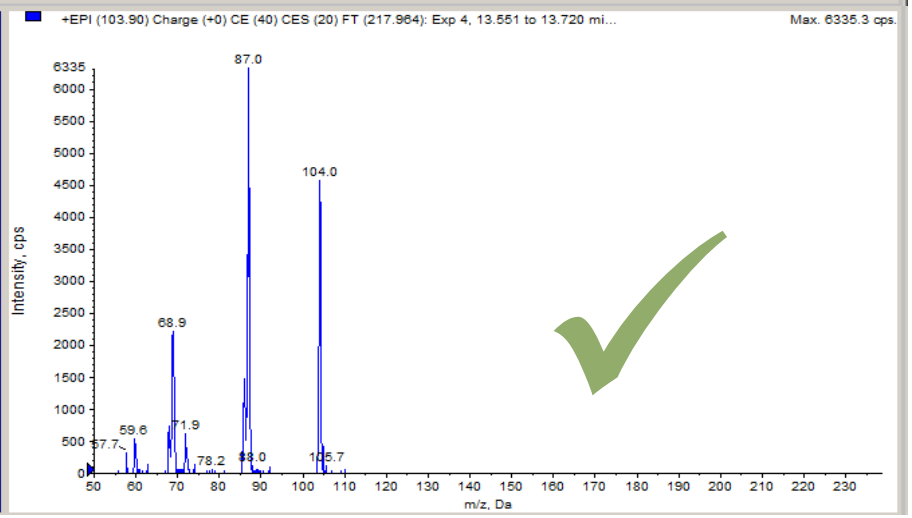


# HILIC-RPLC application II: pharmaceuticals and neurotransmitter



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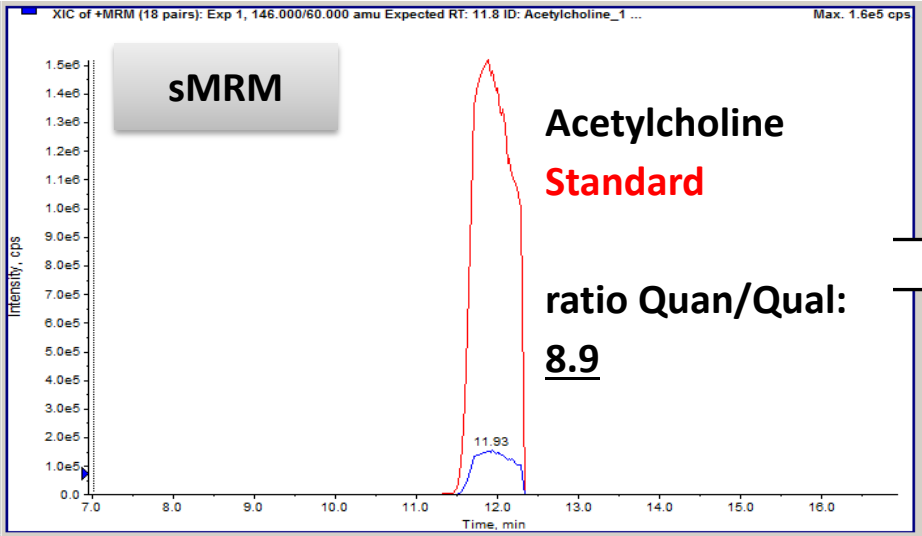
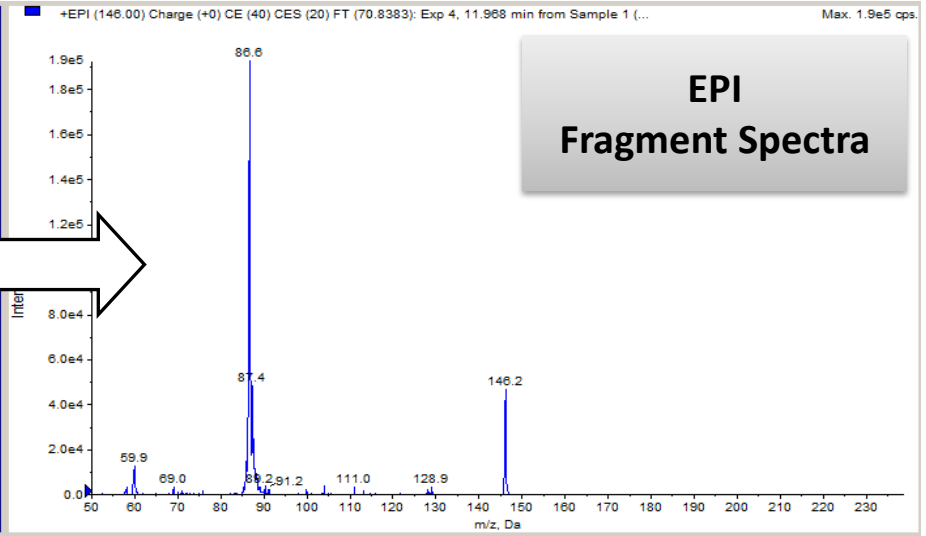
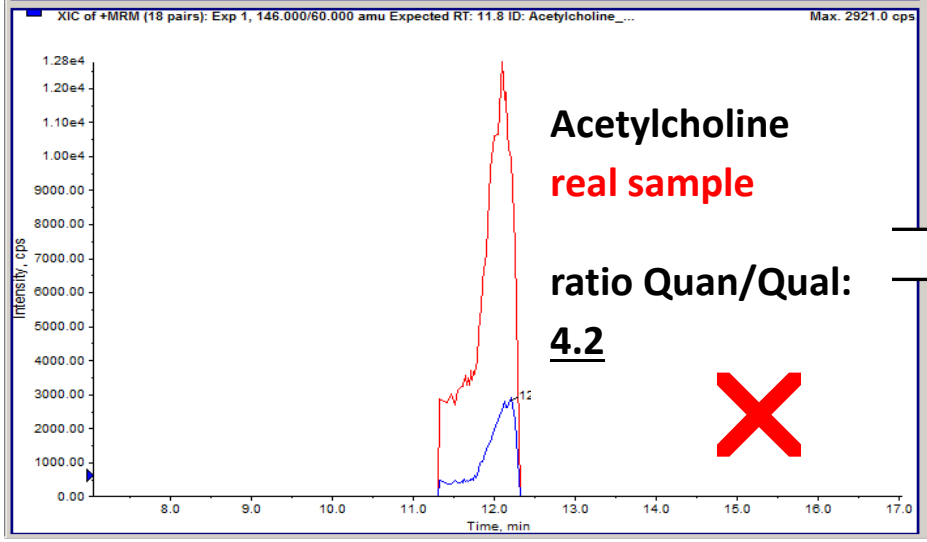
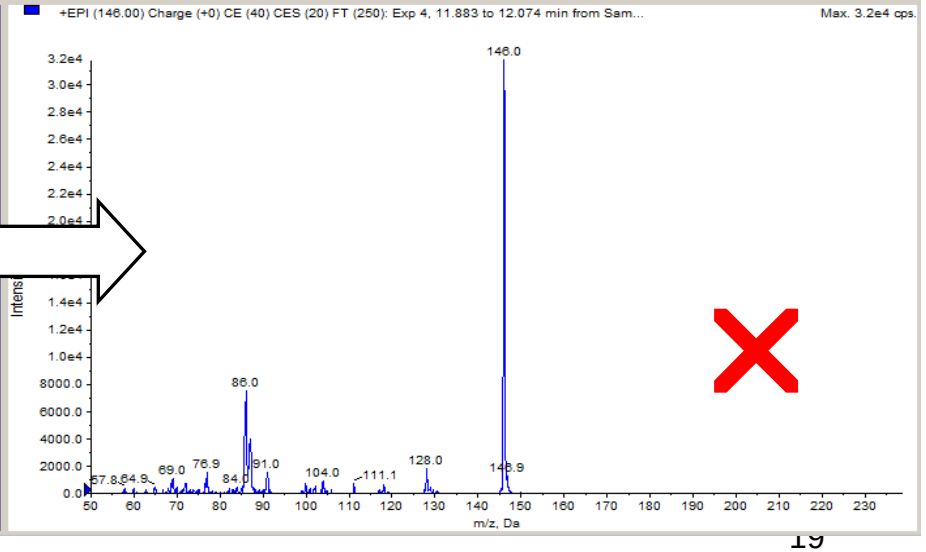
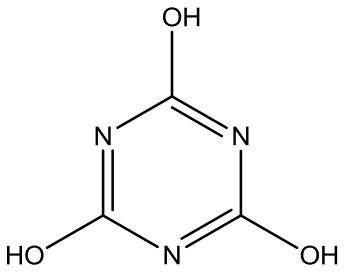


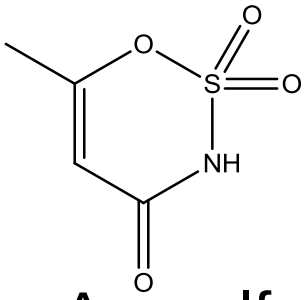



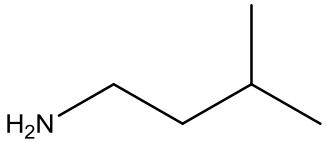
# HILIC-RPLC application III: Sweetener and industrial chemicals, herbicide



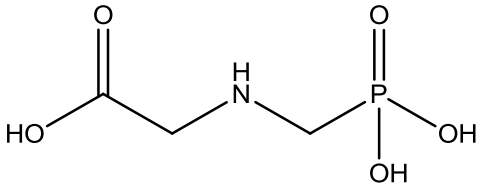

Cyanuric acid



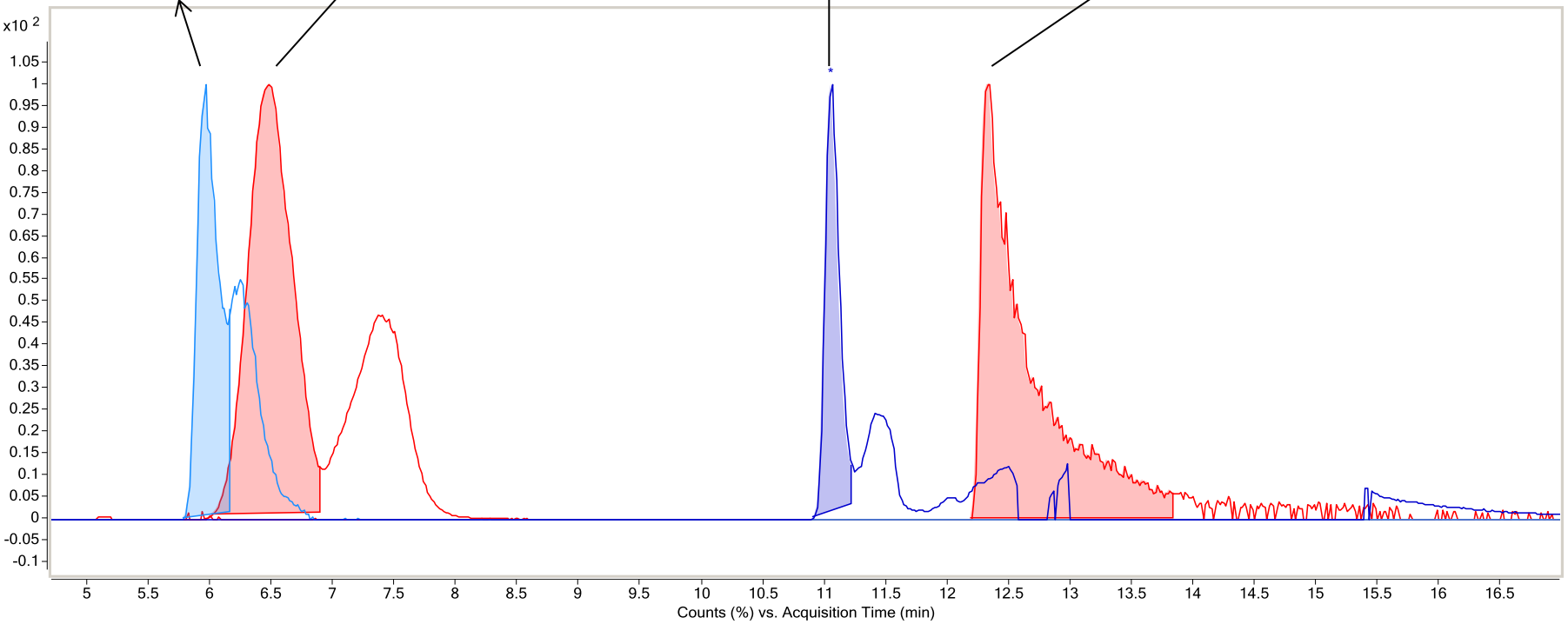

Acesulfam



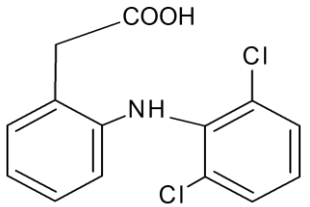

Isopentylamine




Glyphosate



# HILIC-RPLC application IV: An Oxidation Scenario with Diclofenac




**Diclofenac (DCF)**

**BDD electrode**

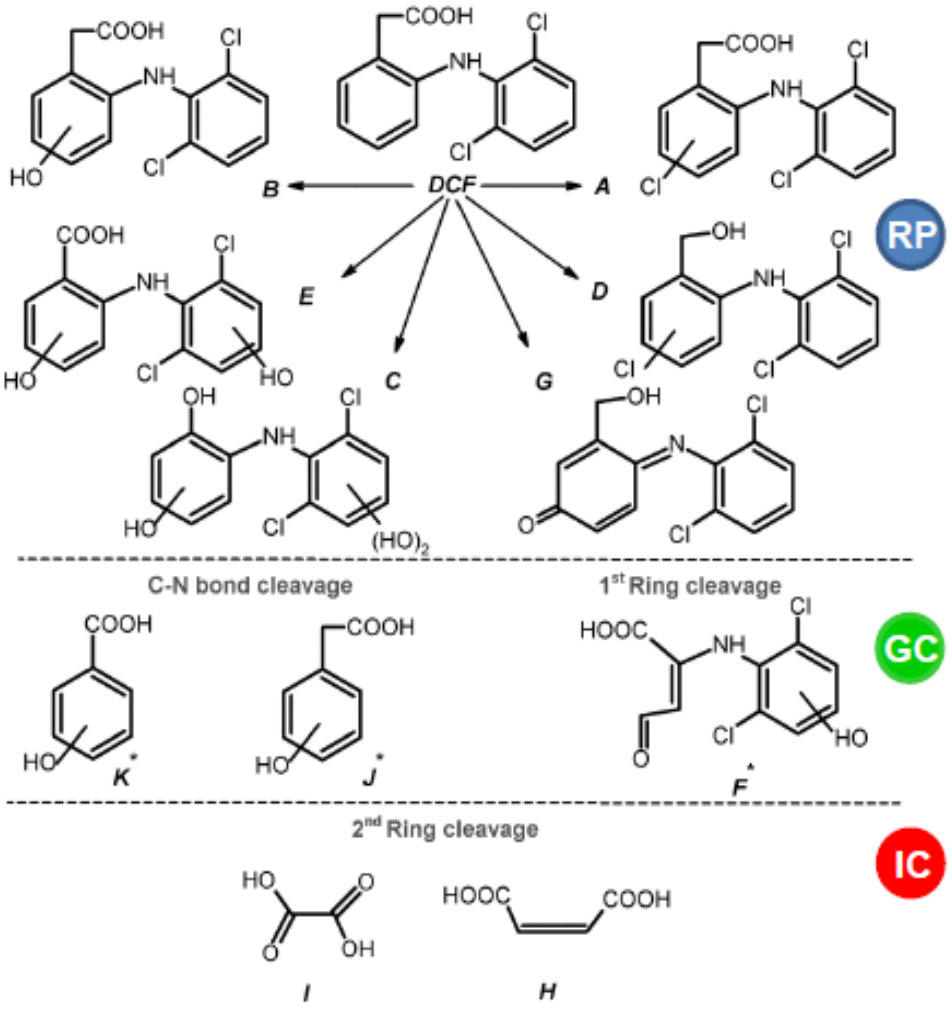

- **MilliQ water**
- **Synthetic hard water**
- **Real wastewater effluent**

**Sample collection: 0-60 min**

Rajab, Greco, et al., *J. Sep. Sci.* 2013.

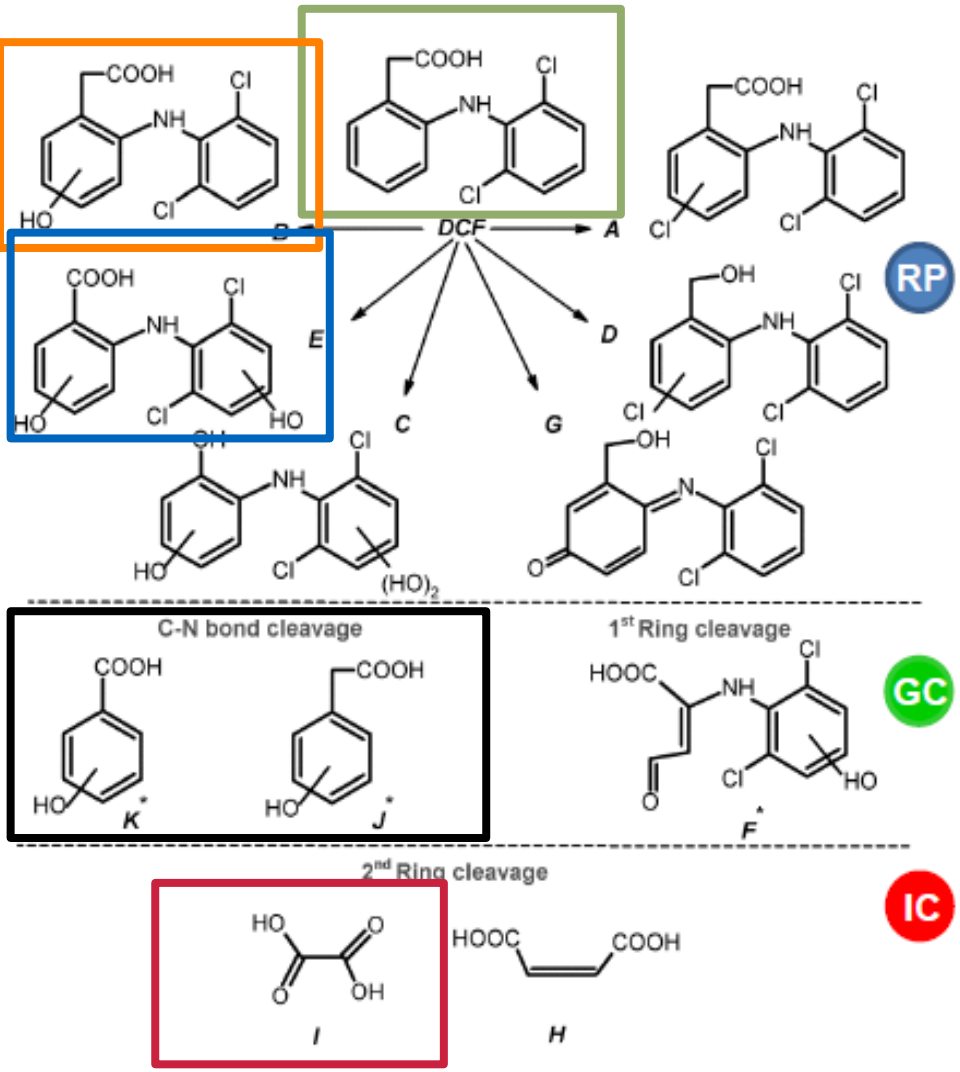
# HILIC-RPLC application IV: Diclofenac oxidation

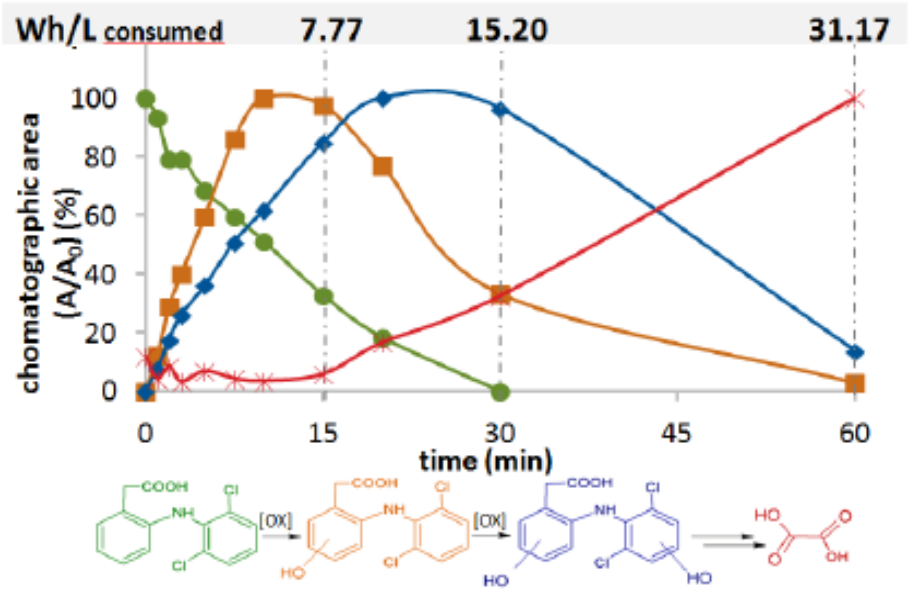
Literature proposed transformation products




Rajab, Greco, et al., *J. Sep. Sci.* 2013.

# HILIC-RPLC application IV: Diclofenac oxidation



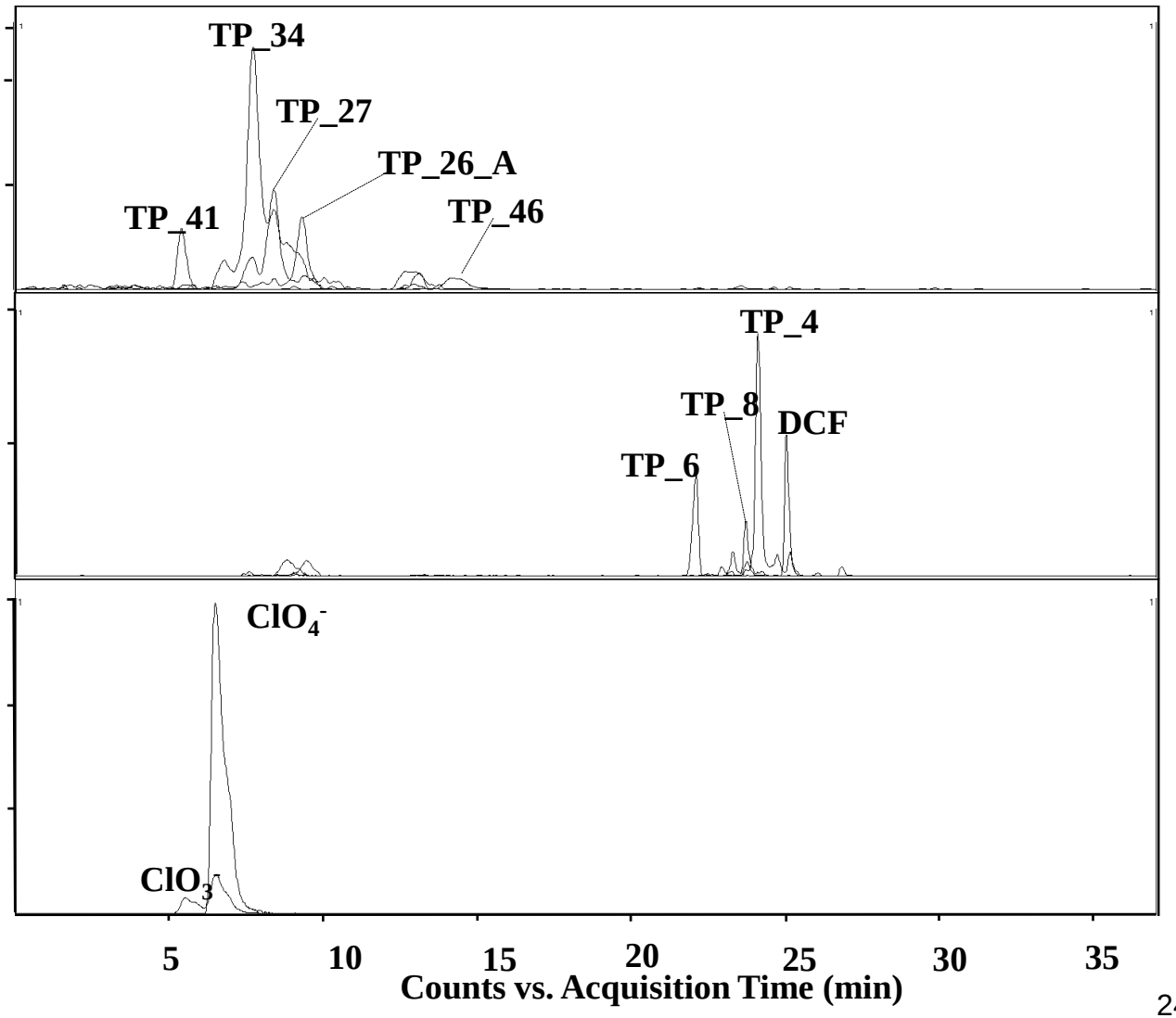




Rajab, Greco, et al., *J. Sep. Sci.* 2013.

# HILIC-RPLC application IV: Diclofenac oxidation

Organic transformation products

Inorganic compounds



# conclusion

- **Understanding of HILIC mechanisms**
- **HILIC valid with logD value < 0**

- **extended polarity with serial RPLC-HILIC coupling**
- **Combination of different chromatographic techniques in just one technique (RP, GC, IC)**

→ A new field of molecules is taped and will give a lot more interesting results

# Thanks...

Dr. Giorgia Greco
Dr. Mohamad Rajab
Sofia Veloutsou
Prof. Dr. Drewes
and the whole team

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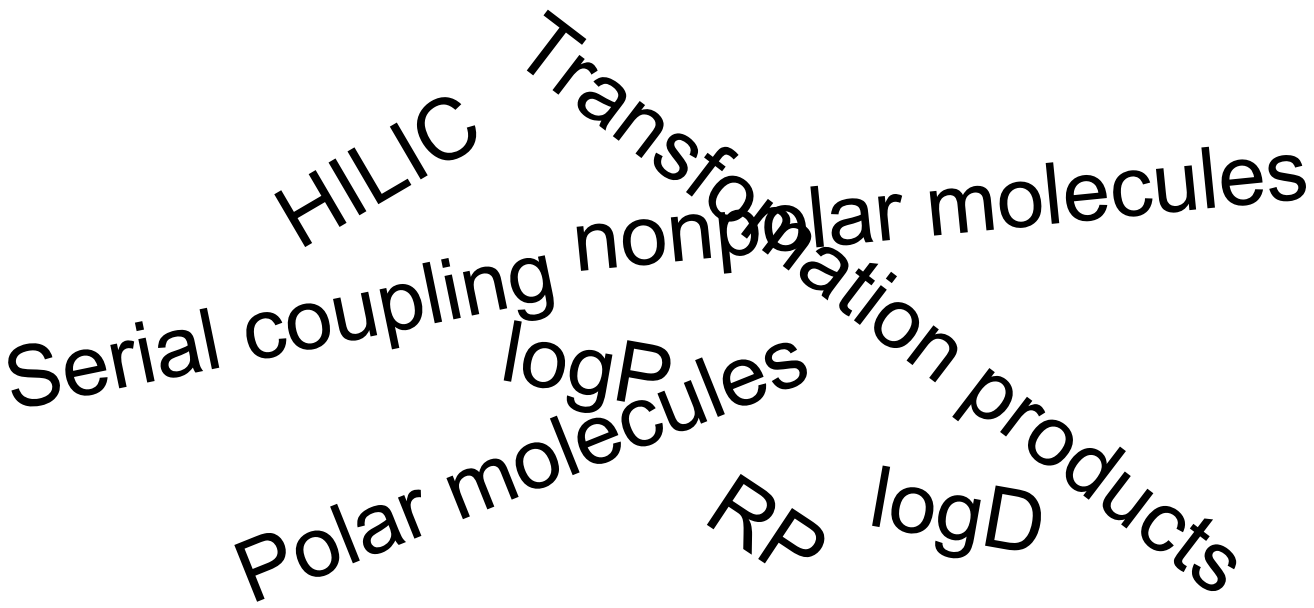
Funded by:
Bundesministerium für Bildung und Forschung

# Thanks...

## And for your attention...




## Any questions???