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

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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 ($P$):
$$\log P = \log \frac{[\text{solute}]_{\text{oct}}}{[\text{solute}]_{\text{wat}}}$$

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

Hydrophilic compounds

Hydrophobic compounds

Log $P < 0$

or

Log $D < 0$

Log $P > 0$

or

Log $D > 0$
Separation of polar and nonpolar compounds

HILIC
hydrophobic interaction liquid chromatography

RPLC
Reversed phase liquid chromatography

NP stationary phases
RP eluents

Typical mobile phase

ACN/H₂O

H₂O/ACN

RPLC vs. HILIC:
• Orthogonal
• Use of the same solvents
• MS compatible
Retention mechanisms

Analyte - Stationary Phase

- ✓ Hydrogen bonding
- ✓ Dipole - Dipole
Retention mechanisms

DISTRIBUTION

Retaining mechanisms:
- At least 2-3% water are essential in the mobile phase!

Retention mechanisms

**ELECTROSTATIC INTERACTION**

**Electrostatic attraction**

- 90% ACN
- Water layer
- Charged stationary phase

**Electrostatic repulsion**

- 90% ACN
- Water layer
- Charged stationary phase
Stationary Phases

HILIC stationary phases

Unmodified bare silica gels

Polar chemically bonded phases

Silica

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

<table>
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<tr>
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<th>Distribution</th>
<th>Electrostatic interaction</th>
<th>Adsorption</th>
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<tr>
<td>Amide</td>
<td>✓</td>
<td>✗</td>
<td>✓</td>
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<tr>
<td>Diol</td>
<td>✓</td>
<td>✗</td>
<td>✓</td>
</tr>
<tr>
<td>Cross-linked Diol</td>
<td>✓</td>
<td>✗</td>
<td>✓</td>
</tr>
</tbody>
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Stationary Phase: ionic

Aminopropyl

\( \text{NH}_3^+ \)

Silica

\( \text{Si} - \text{O}^- \)

\( \text{Si} - \text{OH} \)

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<td>Anion exchange</td>
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<td>pH &lt; 4/5</td>
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<tr>
<td></td>
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<td>pH &gt; 4/5</td>
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<td></td>
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<td>Cation exchange</td>
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</tbody>
</table>

Stationary Phase: zwitterionic

Sulfo betaine (ZIC-HILIC)

- Phosphorylcholine (ZIC-cHILIC)

Orthogonality RPLC and HILIC
RPLC-HILIC coupling

C18 Poroshell
(50 x 3.0, 2.7μm)

Reversed Phase

0.05 mL/min

100%

NH₄OAc 10 mM + 10% ACN

Eluent A

0%

ACN + 10% NH₄OAc 10 mM

Eluent B

ZIC-HILIC
(150 x 2.1, 5μm)

HILIC Phase

0.40 mL/min

100%

100%

100%

0%

ACN

Eluent A

H₂O

Eluent B

Reference solution

Eluent C

0.05 mL/min

RP gradient

Time (min)

B %

0 20 40 60 80 100

0 10 20

HILIC gradient

Time (min)

B %

0 20 40 60 80 100

0 10 20

(ToF)-MS detection
Polarity Extension

**No HILIC**

**Serial coupling of HILIC and reversed phase liquid chromatography**

**Reversed phase liquid chromatography**

**RPLC**

**HILIC**

**RPLC**
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
HILIC-RPLC application II: pharmaceuticals and neurotransmitter

gamma aminobutyric acid?  Acetylcholine?
HILIC-RPLC application II: pharmaceuticals and neurotransmitter

gamma-Aminobutyric acid
Standard
ratio Quan/Qual: 3.6

gamma-Aminobutyric acid
real sample
ratio Quan/Qual: 4.1
HILIC-RPLC application II: pharmaceuticals and neurotransmitter

Acetylcholine
Standard
ratio Quan/Qual: 8.9

Acetylcholine
real sample
ratio Quan/Qual: 4.2

X

X

EPI Fragment Spectra
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)

- COOH
- NH
- Cl

BDD electrode

- MilliQ water
- Synthetic hard water
- Real wastewater effluent

Sample collection: 0-60 min

HILIC-RPLC application IV: Diclofenac oxidation

Literature proposed transformation products

HILIC-RPLC application IV: Diclofenac oxidation

HILIC-RPLC application IV: Diclofenac oxidation

Organic transformation products

Inorganic compounds

Counts vs. Acquisition Time (min)
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 tapped and will give a lot more interesting results
Thanks...

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Thanks...

And for your attention...

Any questions???