UiO : Department of Pharmacy
University of Oslo

Electro Membrane Extraction (EME)

A green, rapid, efficient, selective micro-extraction technique
for clinical samples

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University of Oslo, Norway

Visit us at stand A1.409

extraction
technologies
norway

An introduction to Electro Membrane Extraction

- A microextraction technique derived from liquid-liquid extraction

  - electrophoresis across an oil membrane.

- Invented in 2006 (University of Oslo), to date ~400 scientific papers published.

- Now for the first time available to perform in commercial equipment by Extraction Technologies Norway (ETN AS, Ski, Norway).

Pre-order today!
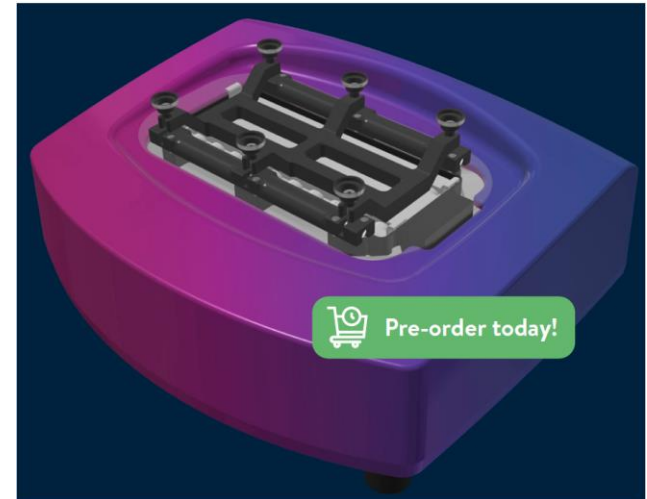
From
Liquid-Liquid Extraction (LLE)

to
Electro Membrane Extraction (EME)

LLE

**Liquid-phase microextraction**

~100 μm thick

EME
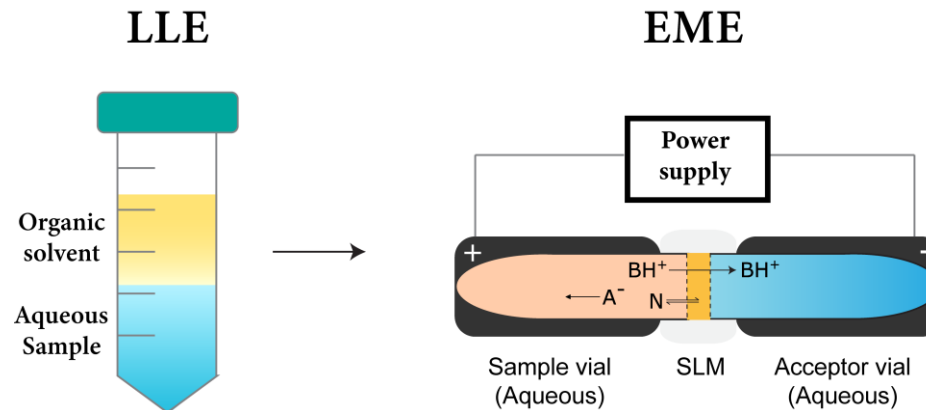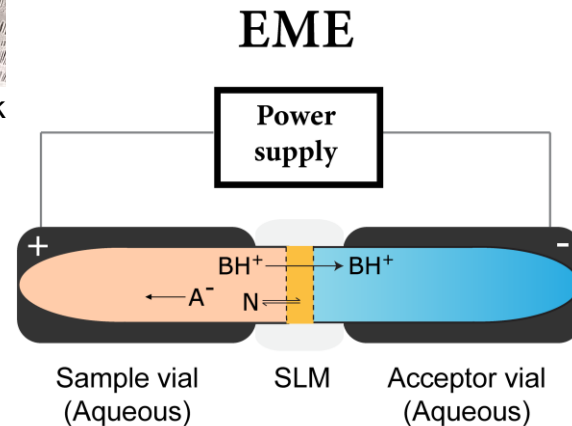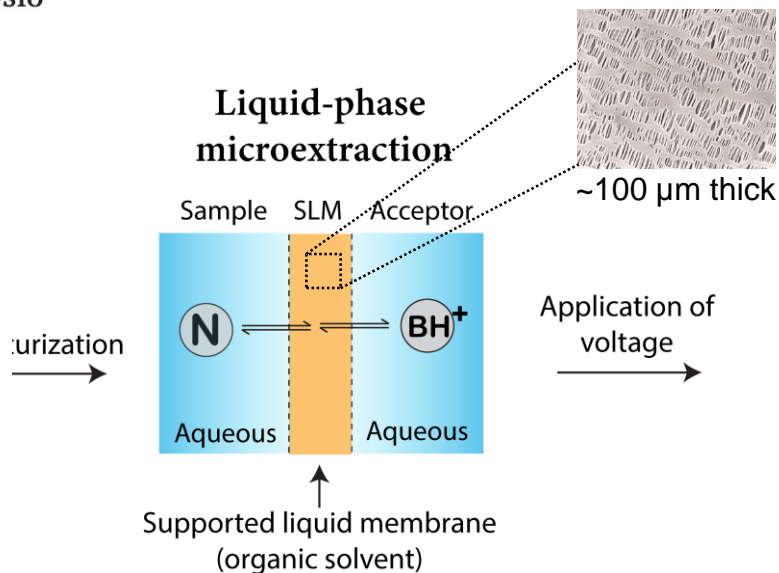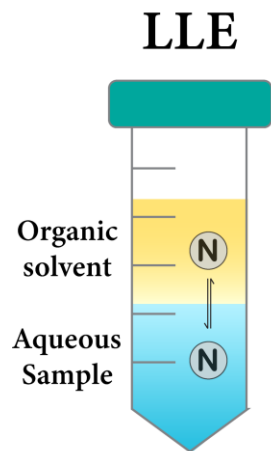
- ✓ Fast (5-10 min)
- ✗ 1-2 mL solvent
- ✗ Not polar analytes
- ✗ Evaporation prior to LC-MS

- ✗ Slow (~60 min)
  - • pH gradient
- ✓ 0.003-0.01 mL solvent
- ✗ Not polar analytes
- ✓ Directly LC-MS compatible

- ✓ Fast (5-30 min)
  - • Electric field driving force
- ✓ 0.003-0.01 mL solvent
- ✓ Polar analytes
- ✓ Directly LC-MS compatible

Advantages of EME

✓ One-step sample preparation

✓ Compatible with very complex samples
  • Whole blood, serum/plasma, urine, cerebrospinal fluid, tissue samples

✓ Selectivity based on analyte charge and hydrophobicity = pure extracts

✓ Complete discrimination of proteins and phospholipids

✓ Non-destructive

✓ Green and safe chemistry
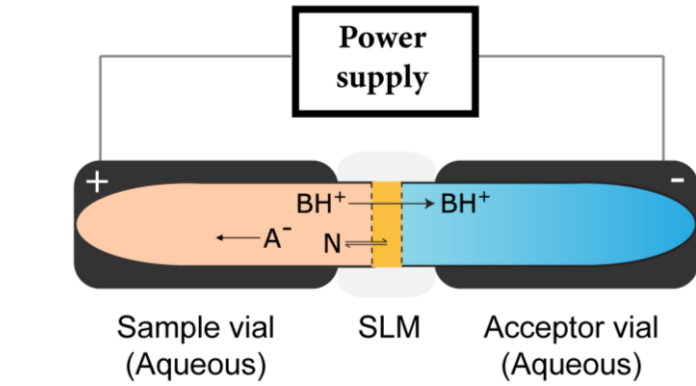
Equipment for EME

Interested in learning more? Visit Hall A1 409
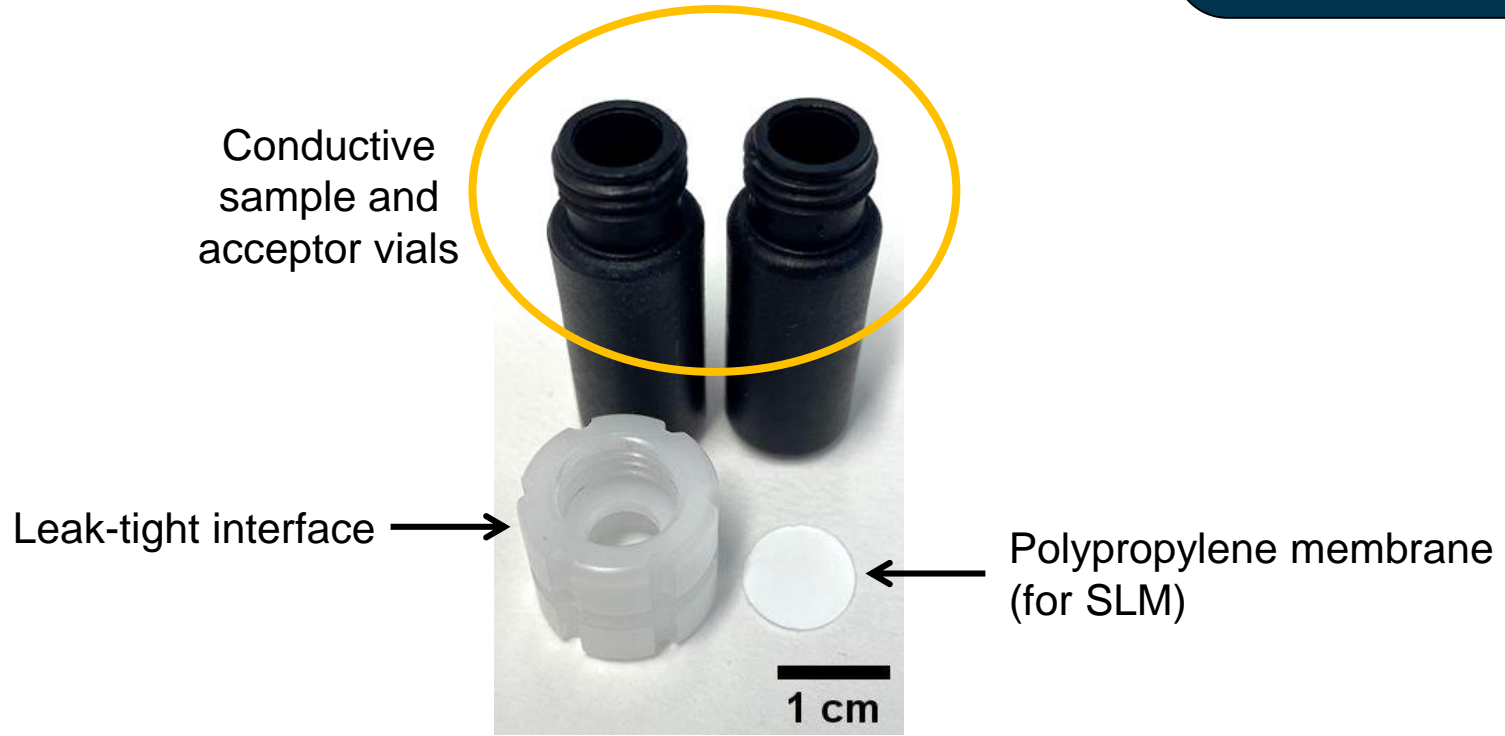
Conductive sample and acceptor vials

Leak-tight interface →

Polypropylene membrane (for SLM)

1 cm

Extraction preparation

Steps:

1. Load ~300 µL sample and acceptor solutions into vials.
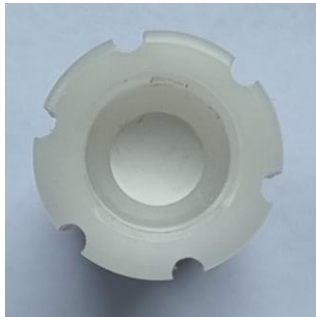2. Place polypropylene membrane in leak-tight interface. Fasten acceptor vial in interface.

Extraction preparation

Steps:

1. Load ~300 µL sample and acceptor solutions into vials.

2. Place polypropylene membrane in leak-tight interface. Fasten acceptor vial in interface.

3. Load 10 µL EME solvent into membrane to prepare supported liquid membrane (SLM).

Without solvent
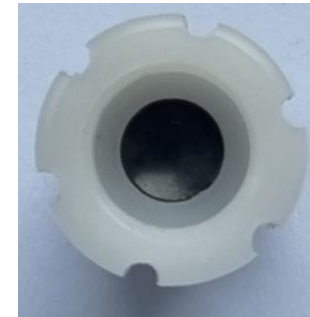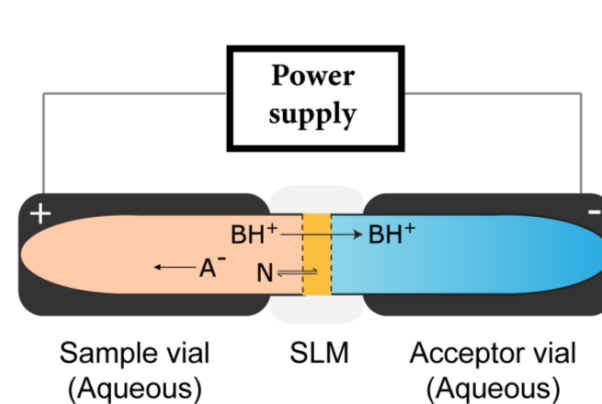
With solvent

Extraction preparation

Steps:

4. Fasten sample vial in interface and place EME unit in shaker.

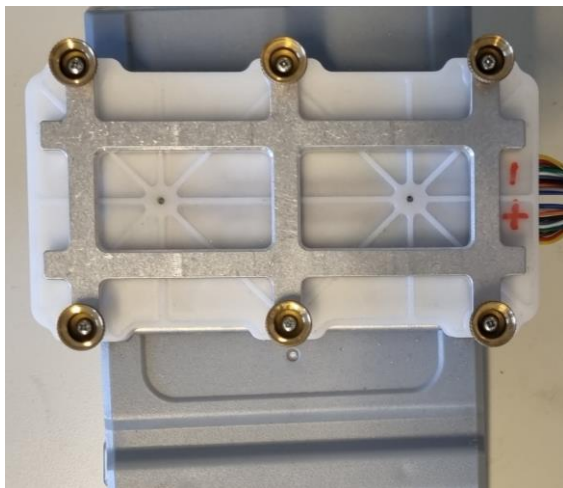5. Close shaker lid, apply shaking and voltage.

Prototype

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Extraction

Horizontal agitation

Prototype
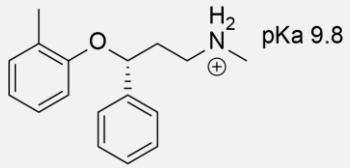
Disassembly
Cap acceptor vial
Place in HPLC autosampler

Practical method development in Electro Membrane Extraction

Analyte considerations
Charge and hydrophobicity

Atomoxetine

pKa 9.8
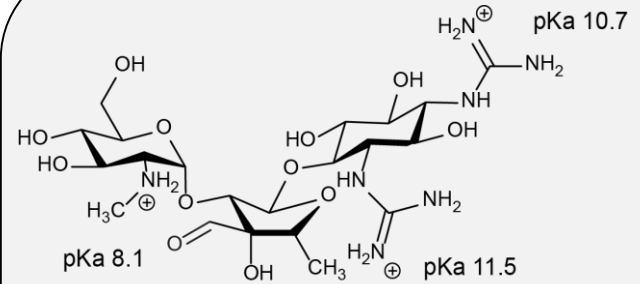
Log P: 3.8
Ionization: Monobasic

pKa 6.4

pKa 4.0

pKa 3.3
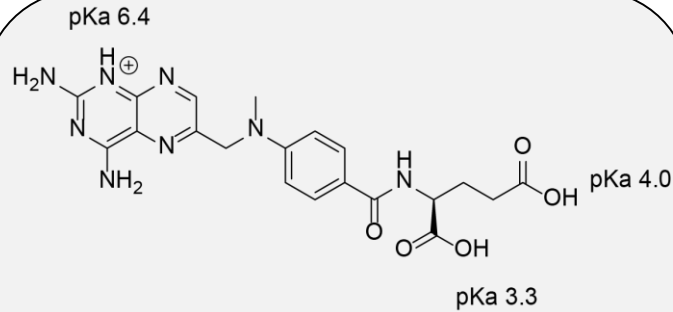
Methotrexate

Log P: -0.4
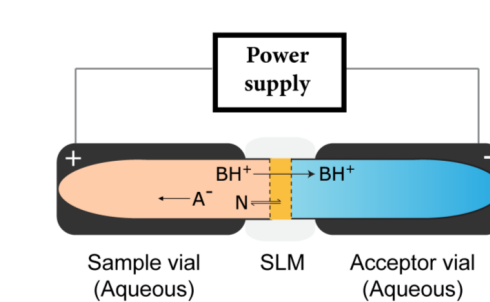Ionization: Zwitterionic

pKa 10.7

pKa 8.1

pKa 11.5

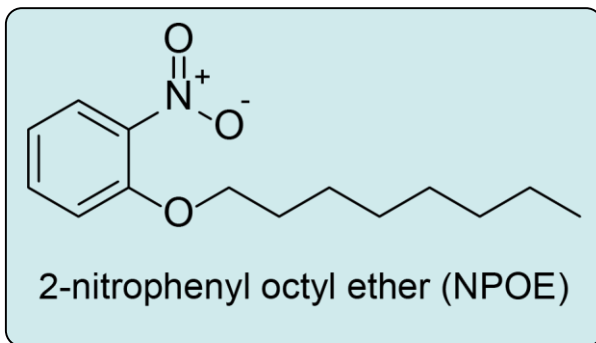Streptomycin

Log P: -7.6
Ionization: Triplebasic

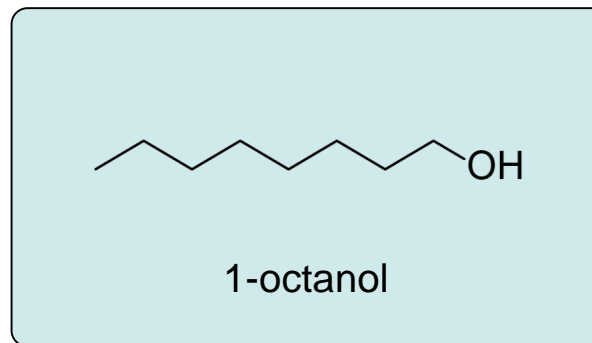# Considerations for EME solvent

Good EME solvents are:
- Water immiscible
- Non-volatile
- Slightly conductive
- Selective based on analyte charge and hydrophobicity

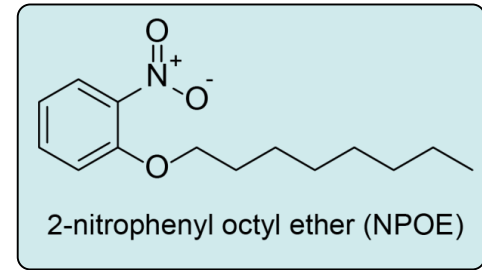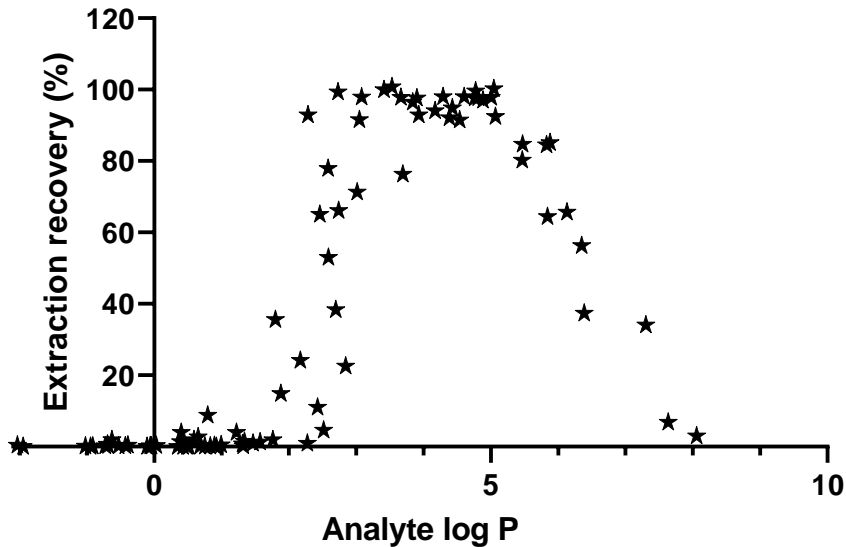**For bases/cations
with log P 2.5-6.0**

2-nitrophenyl octyl ether (NPOE)

**For acids/anions
with log P 1-5**

1-octanol

Considerations for EME solvent

For bases/cations with log P 2.5-6.0

EME of 90 bases

2-nitrophenyl octyl ether (NPOE)

Considerations for EME solvent

For bases/cations with log P < 2.5

6-methylcoumarin   Thymol

Deep eutectic solvent

OR

2-nitrophenyl pentyl ether (NPPE)
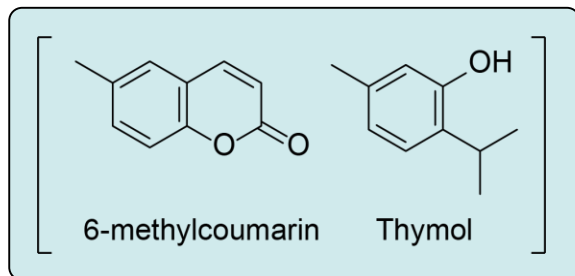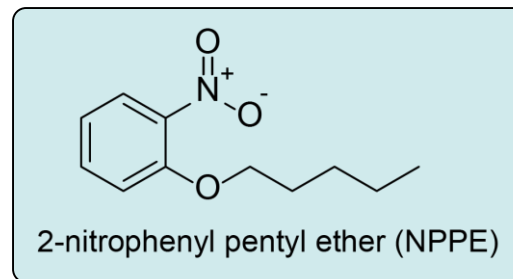
+ ion-pair reagent / transfer catalyst

Di(2-ethylhexyl) phosphate (DEHP)
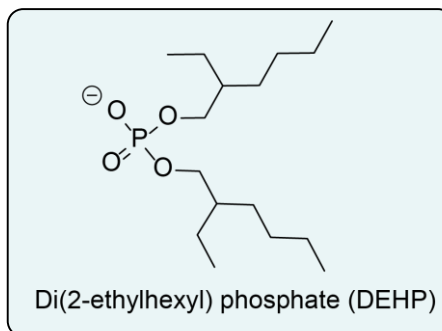
Extraction conditions
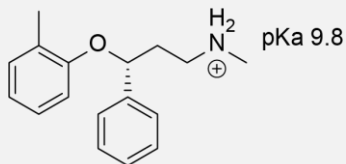
Atomoxetine
pKa 9.8

Log P: 3.8
Ionization: Monobasic

SLM: NPOE

Methotrexate
pKa 6.4
pKa 4.0
pKa 3.3

Log P: -0.4
Ionization: Zwitterionic

SLM: 0.5% DEHP in deep eutectic solvent

Streptomycin
pKa 10.7
pKa 8.1
pKa 11.5

Log P: -7.6
Ionization: Triplebasic

SLM: 75% DEHP in NPPE

Sample and acceptor solutions

- pH value is adjusted to favor analyte ionization
  - pH should be 2-3 units below/above analyte pKa (bases/acids)
- Biological samples are typically diluted 2-5 fold with buffer solution

- Sample pH: may require optimization
- Acceptor solution:
  - 20-100 mM pH modifier
  - MS compatible modifiers:
    - Formic or acetic acid (low pH) or ammonia (high pH)
  - Alternatively:
    - Hydrochloric acid (low pH) or sodium hydroxide (high pH)
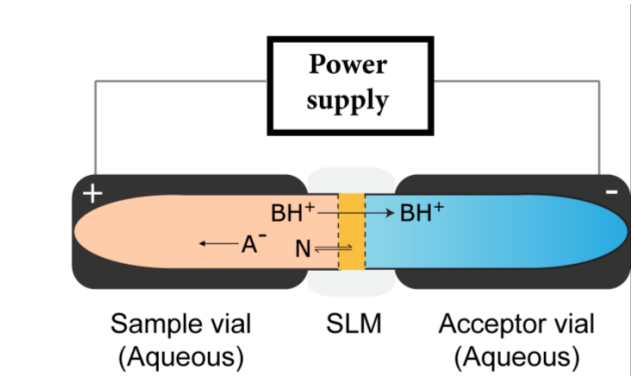
Extraction voltage

- EME is typically performed with 10-50 V applied voltage
- Optimization

Atomoxetine

Methotrexate

Streptomycin
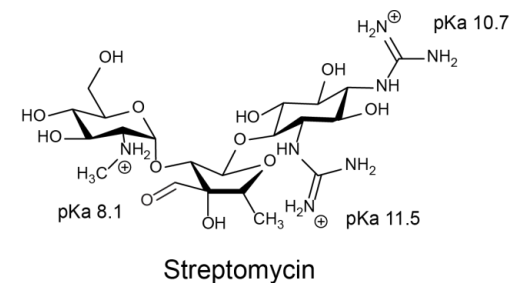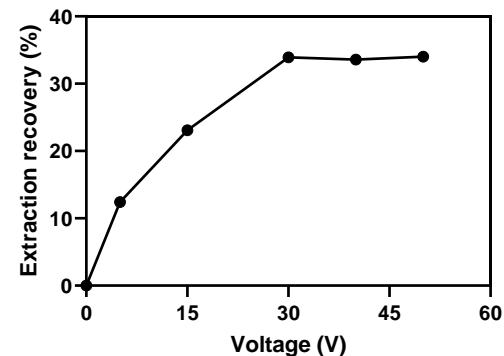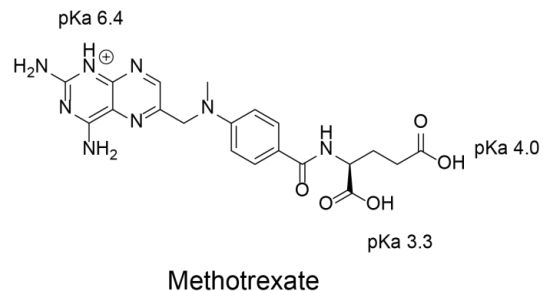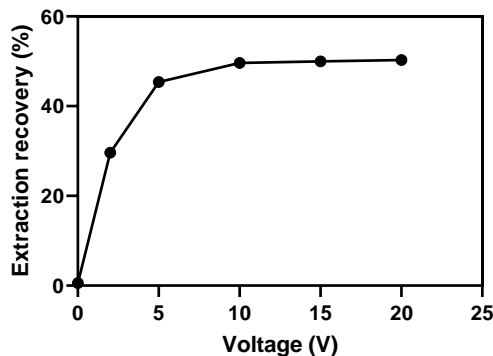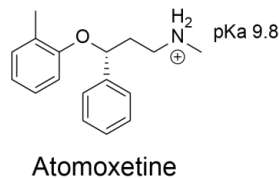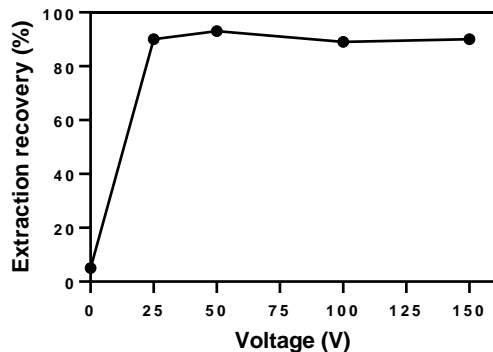
Online process monitoring by extraction current

- Extraction current is a product of ions moving across SLM
- Analogous to HPLC pump pressure – used for process monitoring
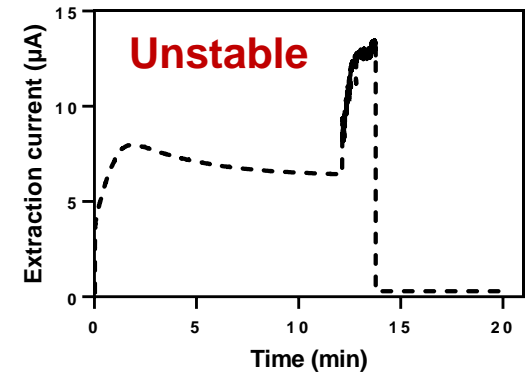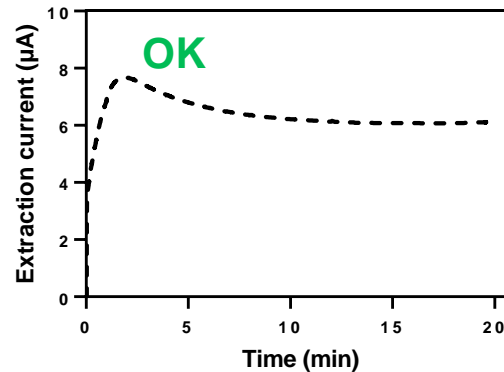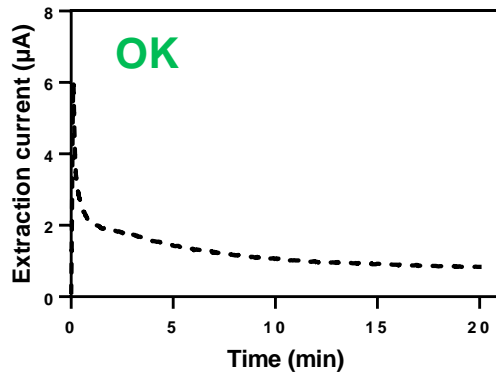
Extraction time

- EME extraction are 5-30 minutes in duration, occasionally up to 60 minutes.

Atomoxetine

Methotrexate

Streptomycin
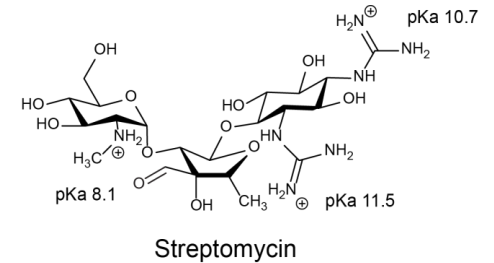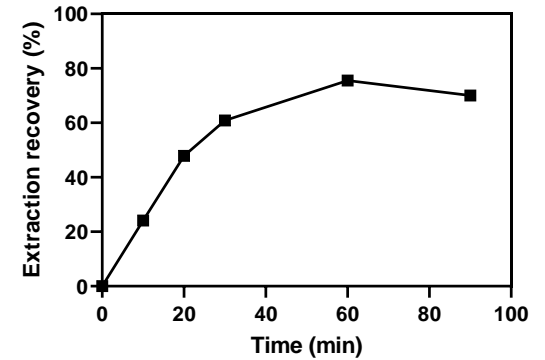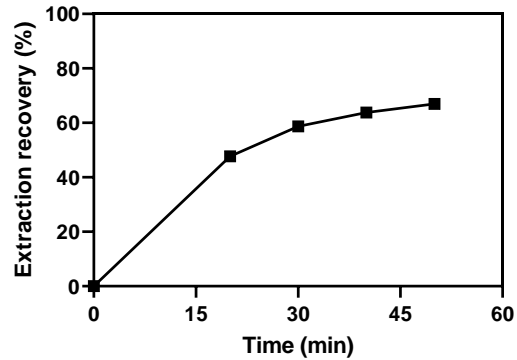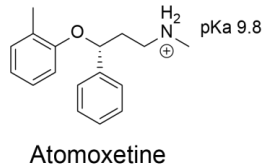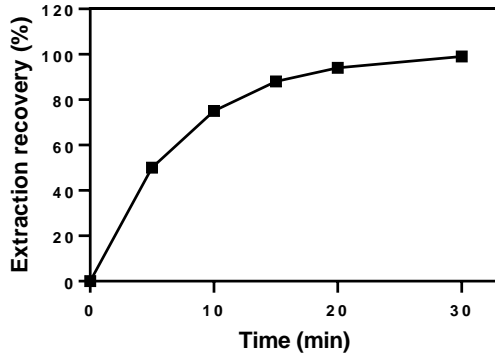
Hyphenation to LC-MS

- EME extracts can be injected directly on LC-MS instruments

  → no need for evaporation + reconstitution

  → clean extracts – reduced matrix effects and down-time

- EME provides good method validation data and low variability.

Validation data for EME-LC-MS/MS determination of Atomoxetine from human plasma samples

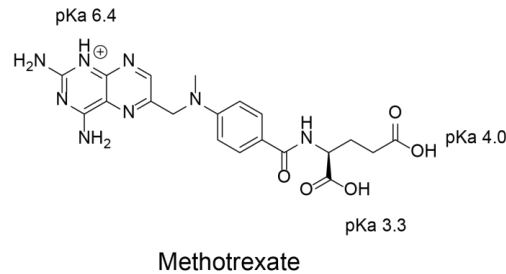| Linear range (nM)	R^2	Recovery (%)	Intra-day precision (%)	Inter-day precision (%)	Accuracy (%)	Matrix effects (%)
40-4000	0.9995	96%	1.7-3.0%	3.0-5.0	1.3-1.8%	101%

Hyphenation to LC-MS

Extracts are free from proteins and phospholipids.

Phospholipid determination after EME of human plasma
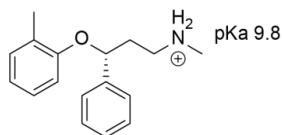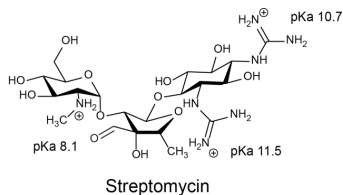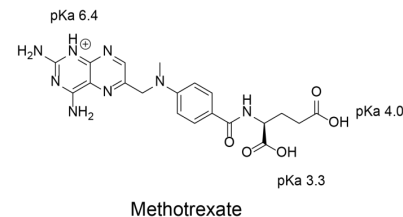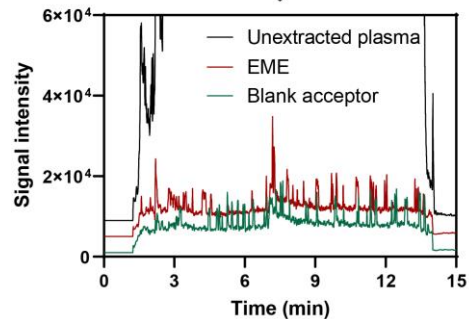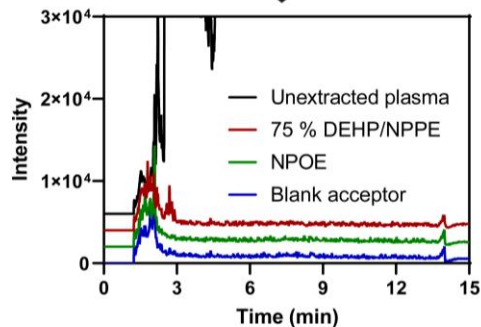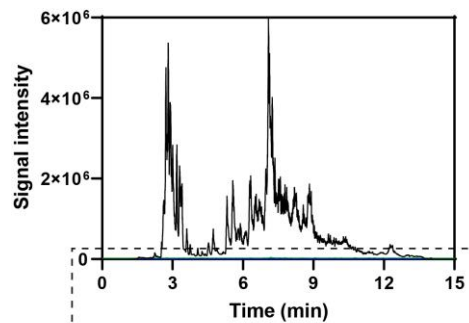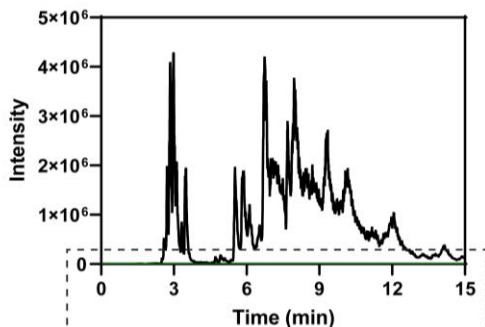
Summary of method development practices

- Consider ionization and hydrophobicity of analyte(s)

- pH adjust sample and acceptor solutions
  - Acidic for basic analytes
  - Basic for acidic analytes

- Select appropriate SLM solvent for analytes
  - Optimization of transfer catalyst (DEHP) for polar analytes.

- Optimize extraction voltage (10-50 V) and time (5-30 min)

- Record extraction current for online process monitoring

Optimize in real sample matrix!

Suitable for DOE!
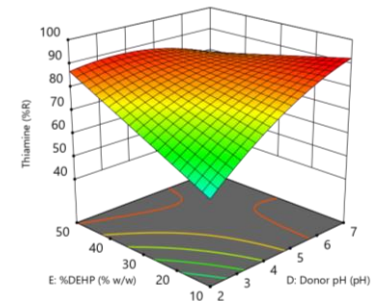
Applications of
Electro Membrane Extraction

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Determination of 12 psychoactive substances in clinical samples

- EME vs routine LLE method at St. Olav University Hospital (Trondheim, Norway)

Procedure:
1. Sample: 100 µL human serum added 25 µL internal standard solution and 175 µL 20 mM formic acid

2. EME:
   - 10 µL NPOE as SLM
   - Acceptor solution: 20 mM formic acid
   - Extraction at 50 V for 15 minutes

3. Acceptor solution analyzed by UHPLC-MS/MS (3 min).

Determination of 12 psychoactive substances in clinical samples

- Comparison of EME and routine LLE method at St. Olav University Hospital (Trondheim, Norway)

  Conclusions:
  - EME method compliant to FDA validation guideline requirements

  - EME performance matched routine LLE method

  - EME simplifies sample preparation – eliminating protein-precipitation, phospholipids removal, filtration, evaporation and reconstitution steps.
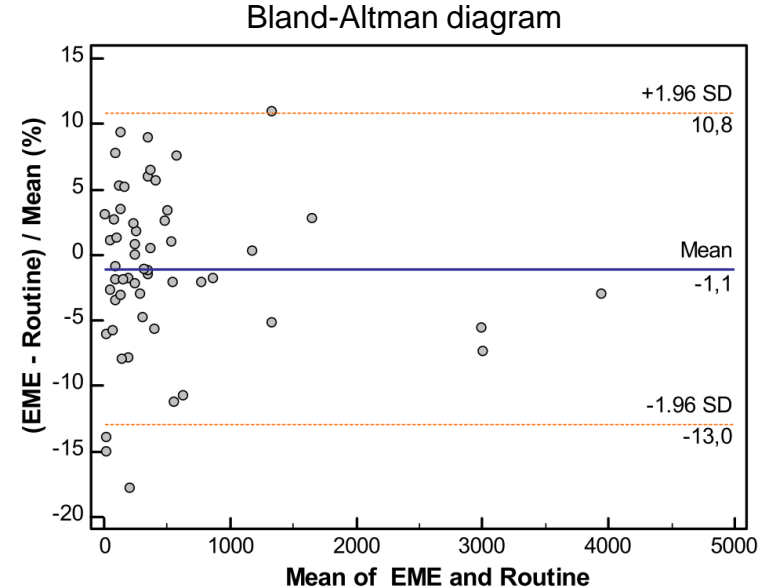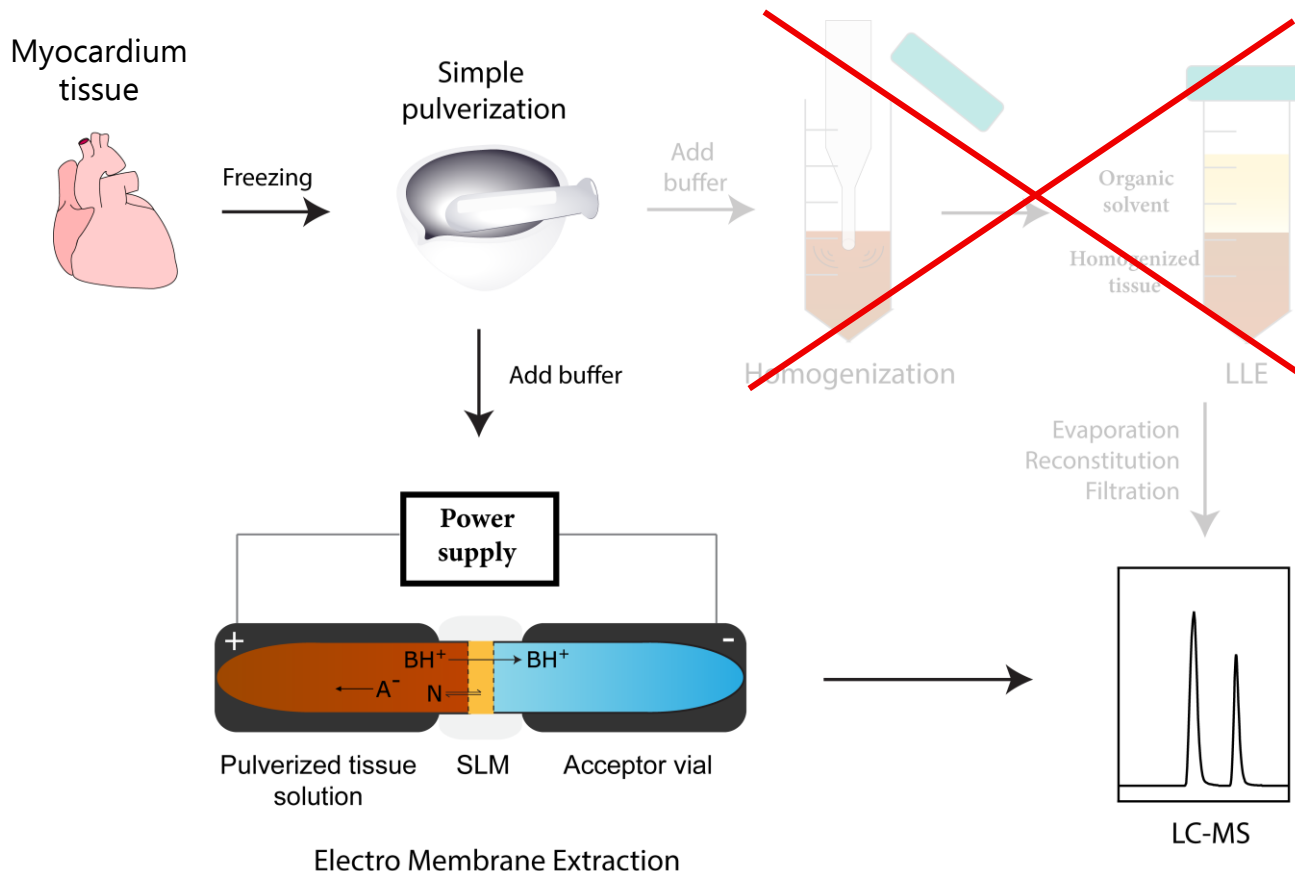
Bland-Altman diagram

Extraction of pharmaceuticals from tissue samples

UiO **: Department of Pharmacy**
University of Oslo

Extraction of pharmaceuticals from tissue samples

Determination of anti-cancer agent and metabolite in rabbit tissue

Procedure:
1. 25 mg pulverized tissue added to 200 μL 0.5 M formic acid + 12.5% methanol

2. EME:
   - DEHPi as SLM
   - Acceptor solution: 0.5 M acetic acid
   - Extracted at 15 V for 25 minutes

3. Acceptor solution analyzed by UHPLC-MS/MS

EME compared to reference LLE method

Daunorubicin   pKa 10.0

Daunorubicinol   pKa 10.0
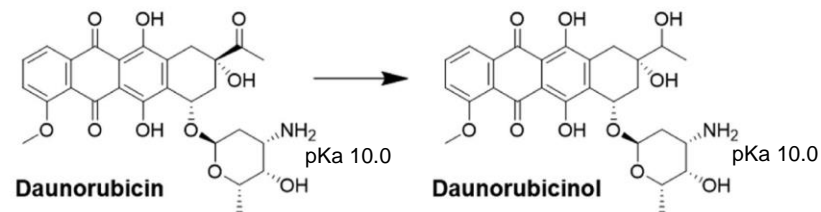
UiO **: Department of Pharmacy**
University of Oslo

Extraction of pharmaceuticals from tissue samples

Determination of anti-cancer agent and metabolite in rabbit tissue

Conclusions:

- EME required fewer steps of sample prep than LLE.
  - No benefit of additional homogenization for EME.

- Improved recovery, sensitivity and sample clean-up with EME.
  - Elimination of phospholipids with EME (unlike LLE).

Interested in learning more? Visit Hall A1 409
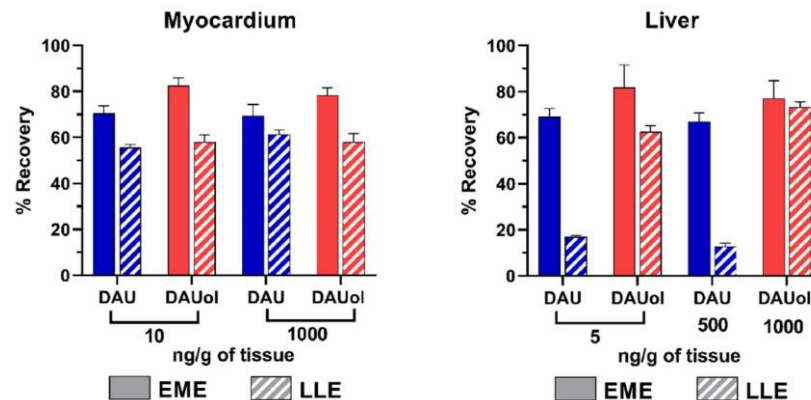
Summary

Electro Membrane Extraction (EME) offers green, rapid and selective extraction of clinical samples…

… but, EME has previously also been applied for many other extraction applications:

- Peptides
- Endogenous metabolites
- Metals and heavy metals
- Salt ions
- Preparative extractions
- Principally, any molecule with a charge can be extracted with EME!