Sample Preparation Techniques for the isolation of drugs from biological fluids.

By Elena Gairloch

Guest Speaker: Dr. Xiangyu Jiang, Associate Director in Chemistry at Covance in Madison, WI.

Biotage

Relative Selectivity

Non selective

Dirty extracts

- Protein precipitation
- Traditional Non-selective resin SPE (hydrophobic only)
- Liquid-liquid extraction
- Mixed-mode SPE - resin based sorbents
- **Resin-based, EVOLUTE ABN**
- C18 silica-based SPE
- C8 silica-based SPE
- C2 silica-based SPE
- **Supported Liquid Extraction, SLE+ or HM-N**
- Ion exchange SPE, silica-based sorbents
- Mixed-mode SPE, silica-based sorbents
- Immunoaffinity, MIPs

Highly selective

Clean extracts

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What is EVOLUTE ABN?

- Extends the range of acidic, basic and neutral compounds that can be extracted using a single generic method, increasing method development productivity
- Has an optimized pore structure which reduces the extraction of endogenous matrix components, and leads to cleaner extracts that give fewer matrix effects in LC-MS/MS analysis
- Has optimized physical and chemical characteristics that improve reliability of SPE procedures
- Has no secondary interactions, allowing the use of pure organic elution solvent to give high recoveries of very low drug concentrations

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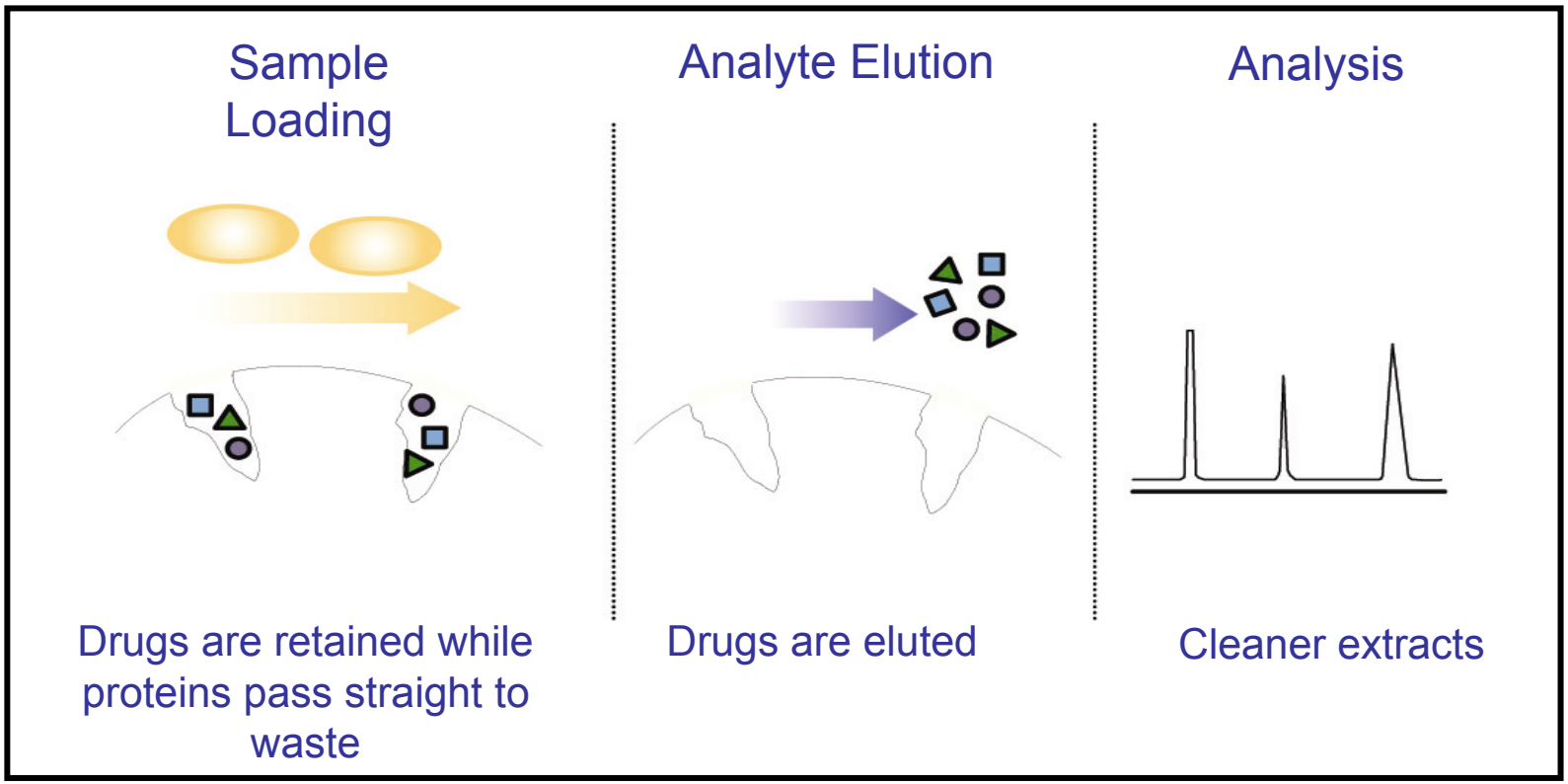
EVOLUTE: Developed to Give Cleaner Extracts



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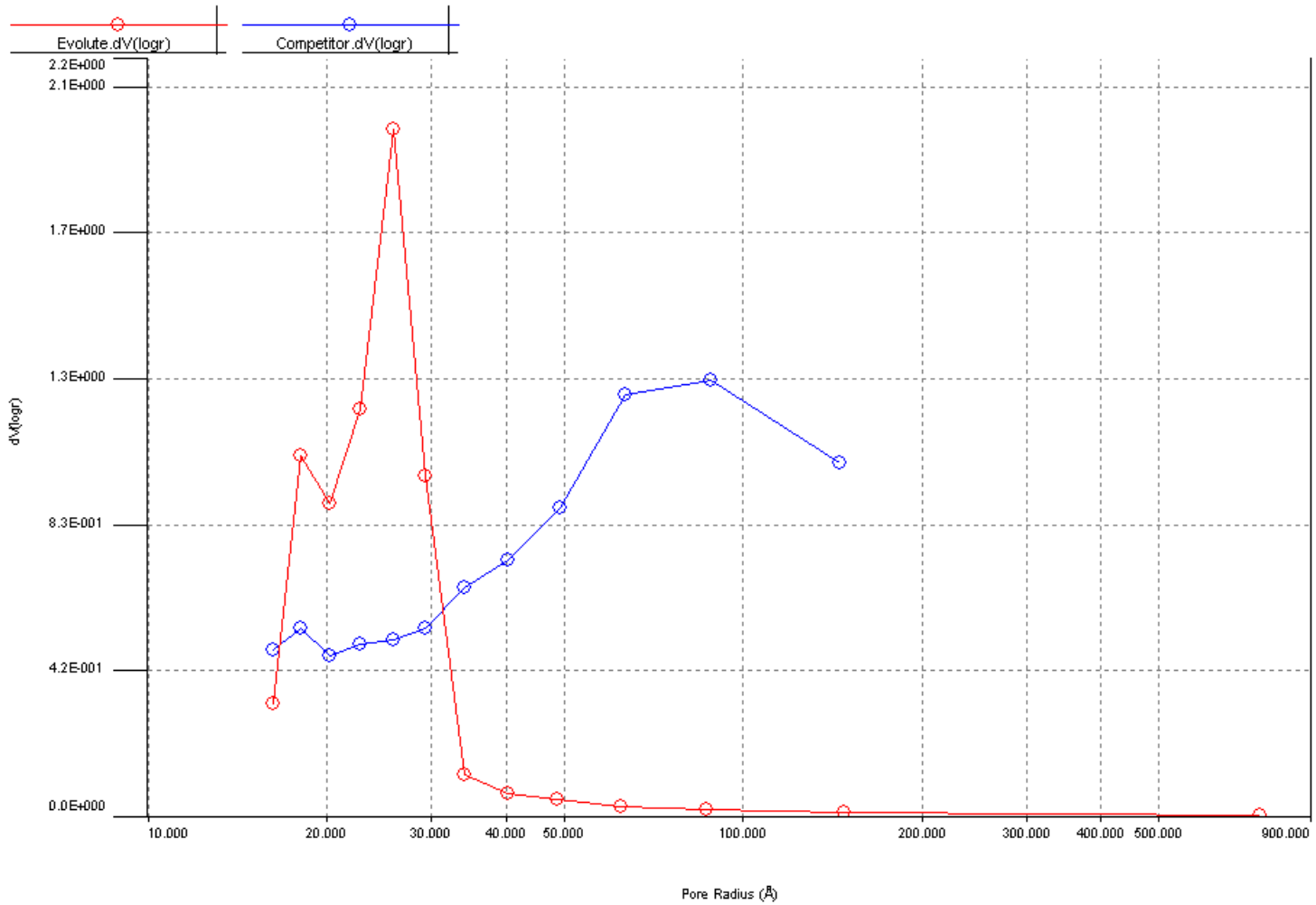
Comparison of Pore Size Distribution



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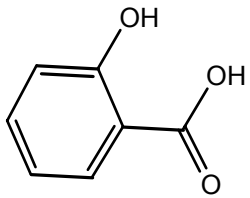
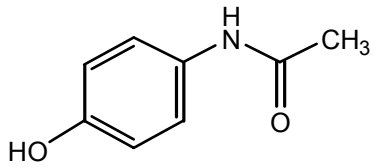
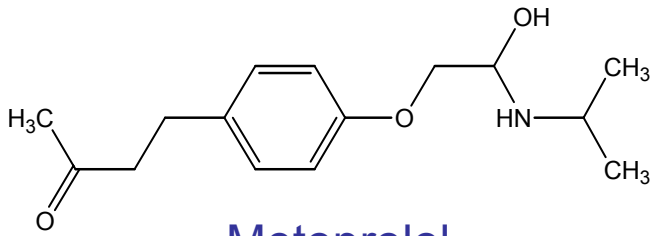
Compound Diversity



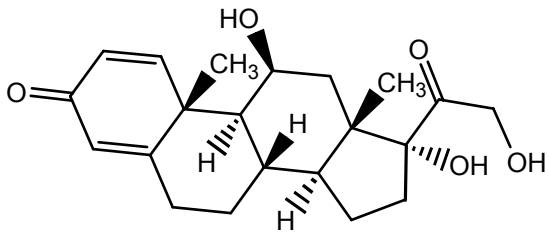
Salicylic acid



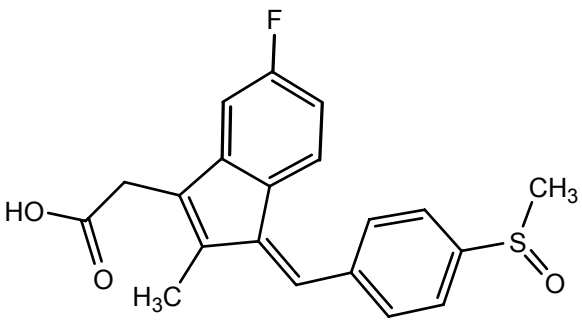
Acetaminophen



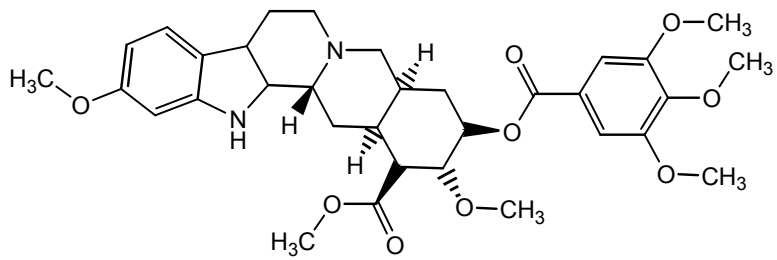
Metoprolol



Prednisolone



Sulindac



Reserpine

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Probe Samples

- Pooled human plasma spiked at 5-50 pg/µL with probe analytes
- Concentration of individual analytes determined by detection limit of LC-MS/MS system

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EVOLUTE ABN Generic Method

Method shown is optimised for 25 mg formats

1.	Pre-treatment	Dilute sample 1:3 (v/v) with 1% formic acid
2.	Conditioning	Methanol (1 mL)
3.	Equilibration	0.1% formic acid (1 mL)
4.	Sample load	400 µL- 2 mL diluted plasma
5.	Interference wash	Water : methanol (95:5, v/v, 1 mL)
6.	Elution	Methanol (500 µL)

Evaporate and reconstitute as necessary for analysis

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Method Comments…Continued

- Neutral pH can also be used
  - E.g. for acid labile compounds
  - Recommend use of 0.1 M ammonium acetate for sample dilution
- 10 mg formats
  - Simple scale down
  - Steps 2,3,5: 500 µL
  - Elution 200 µL (2 x 100 µL)

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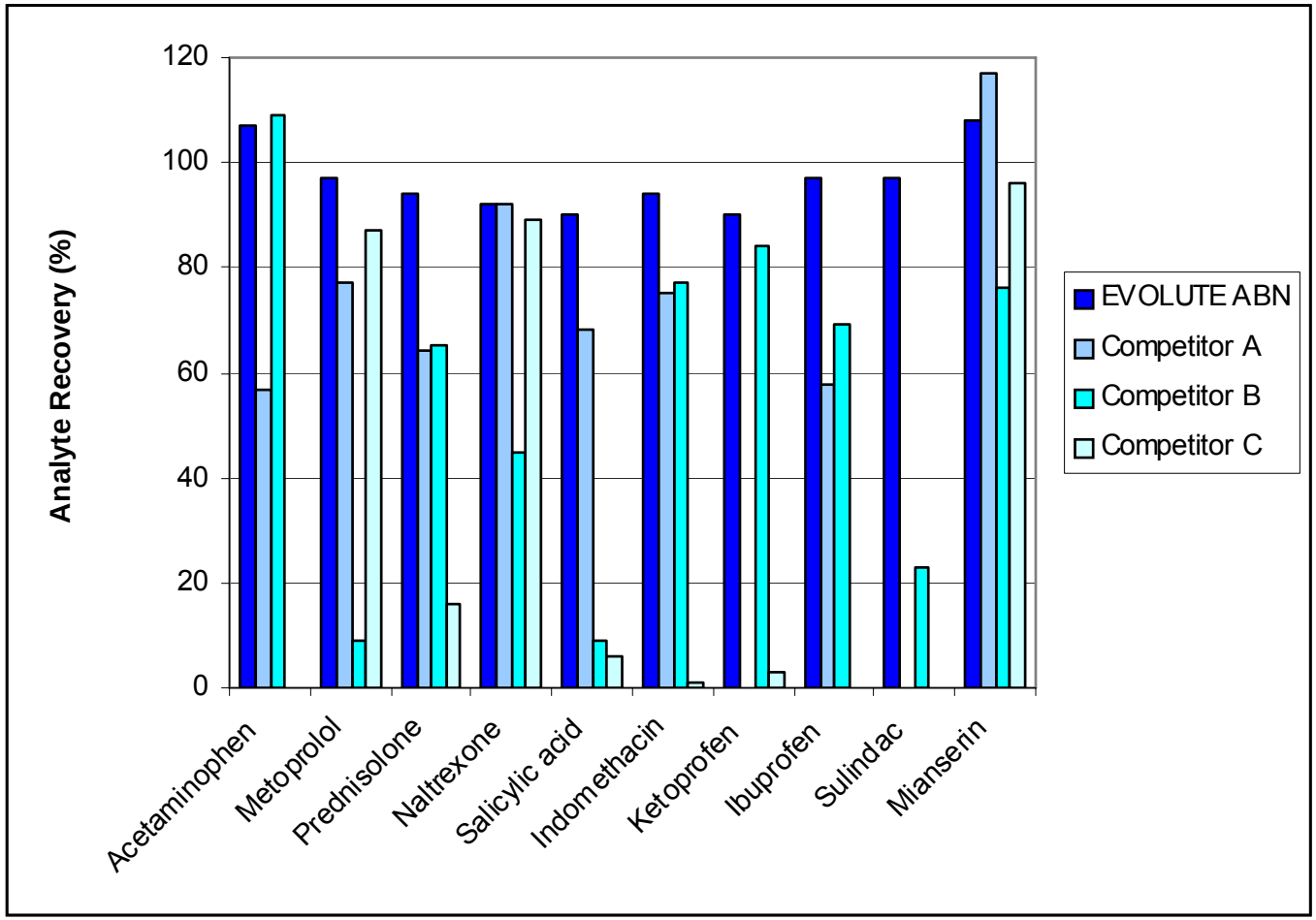
Comparison of Analyte Recoveries



Sample: plasma spiked at 5-50 ng/mL. Methodology used: manufacturer's published generic methods.

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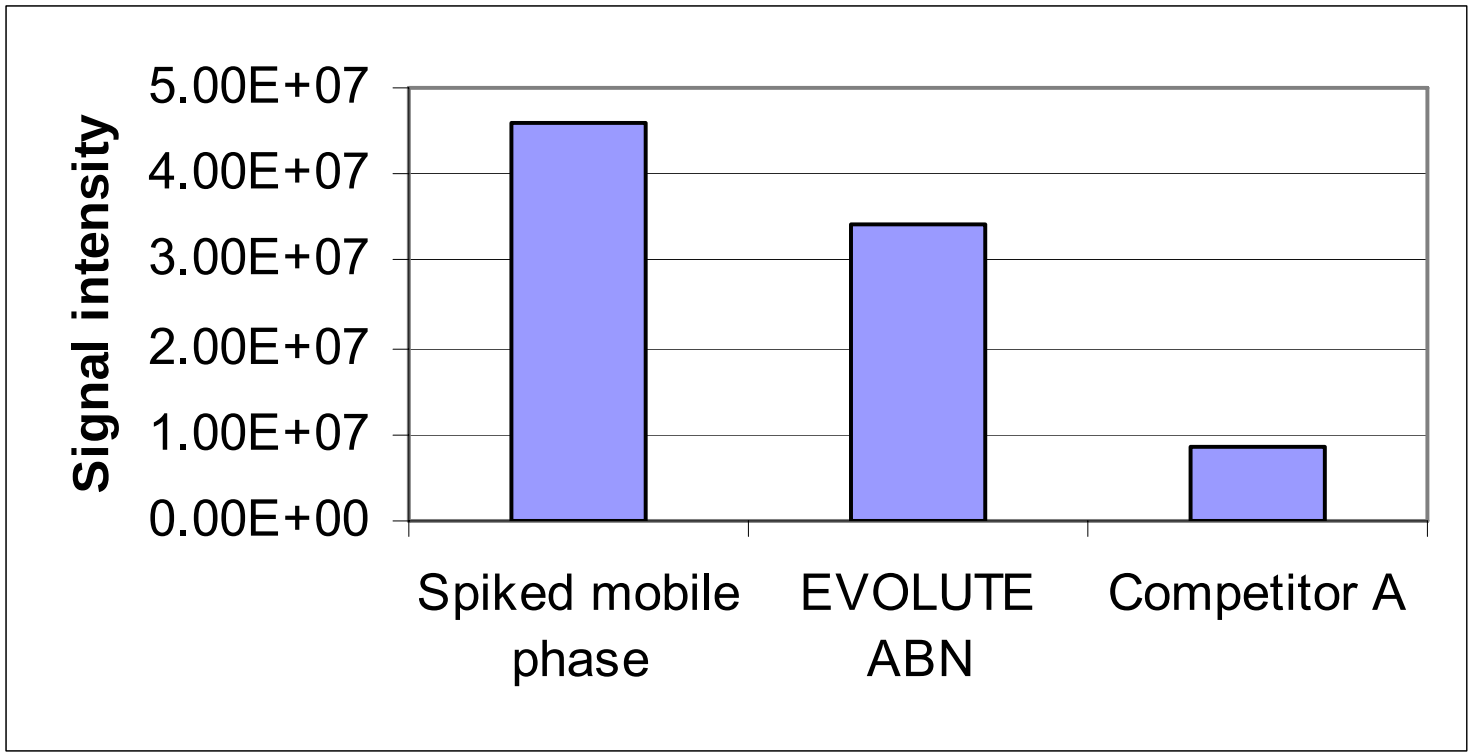
Typical Results



EVOLUTE delivers 4 times improvement in s/n compared to a competitor resin

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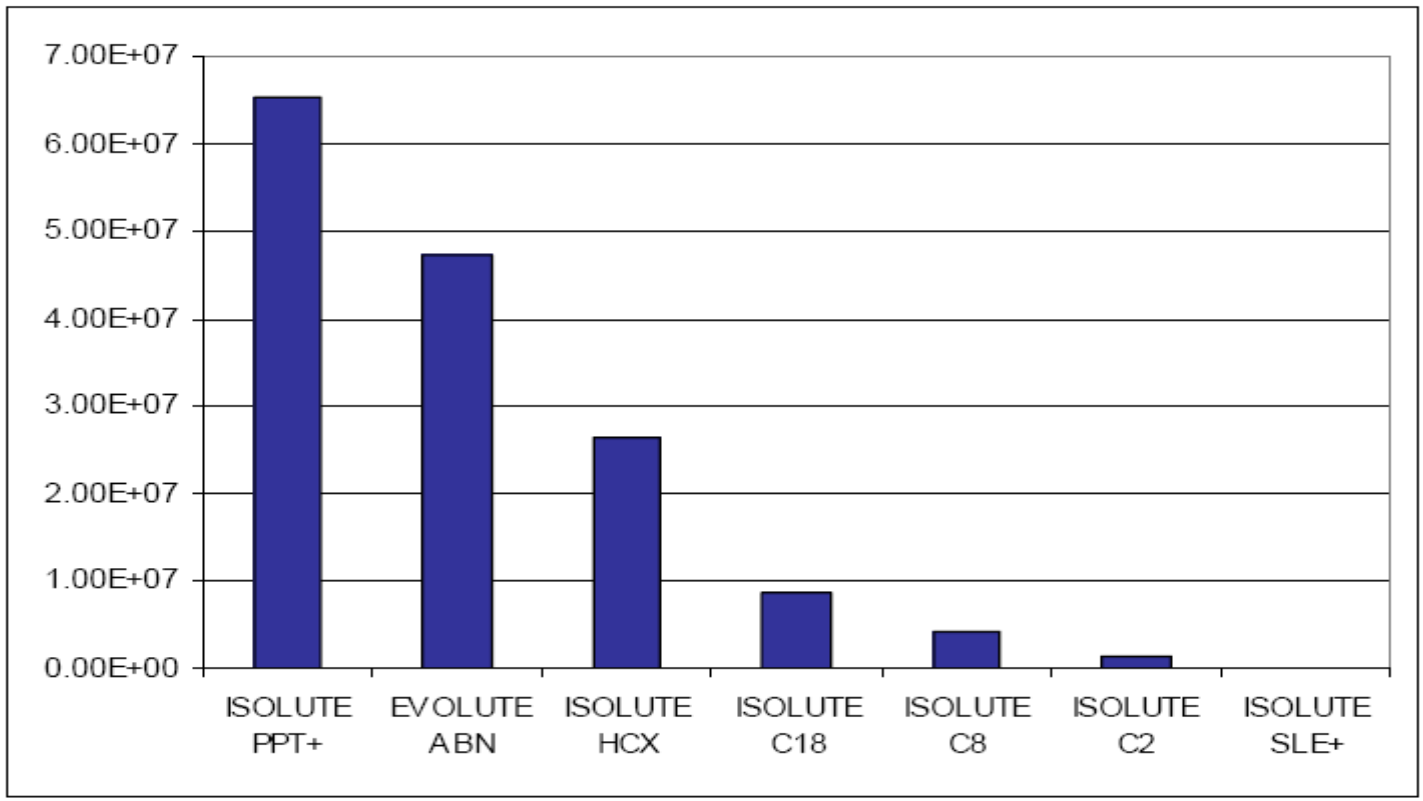
Relative Extract Cleanliness: Phospholipid Removal



Blank pooled human plasma: Area count (TIC, 5.5-8.0 minutes) for residual selected phospholipid ions in human plasma prepared using the various sample preparation techniques. ISOLUTE HCX, a mixed-mode sorbent, gave relatively high phospholipid content, despite the rigorous interference elution regime possible. This is believed to be due to interactions of the zwiterionic phospholipids with both the non-polar and cation exchange functional groups. Further work will investigate this result.

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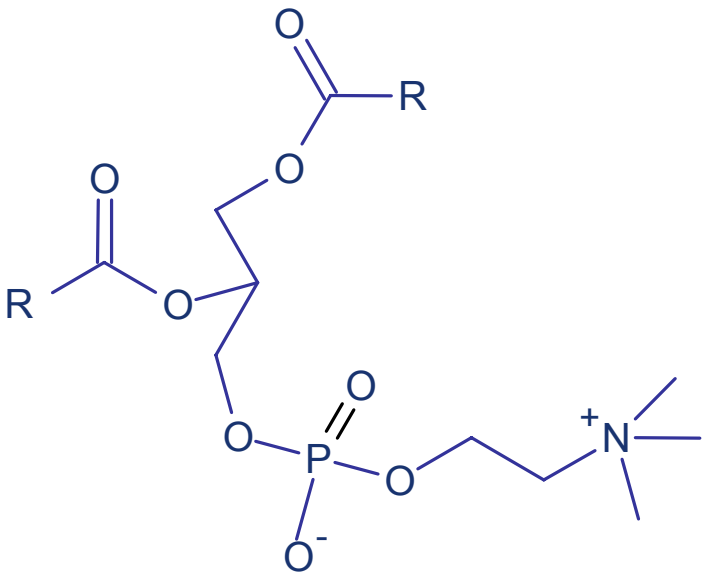
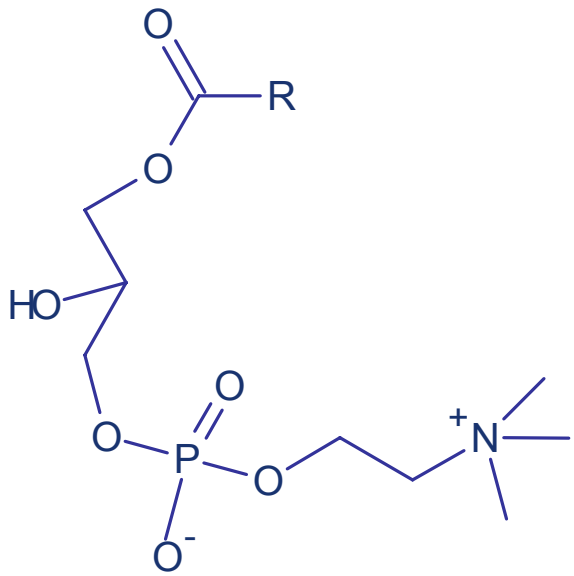
Phospholipid Structures



Phosphatidylcholine



Lysophosphatidylcholine

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Phospholipid Removal by SLE+

- Sample: Blank human plasma (100 µL) was diluted 1:1 with various pH buffers prior to loading onto the ISOLUTE SLE+ Supported Liquid Extraction Plate. The buffers included in this study were;
  - 1% (v/v) formic acid aq, , pH 3.17
  - 0.1% (v/v) formic acid aq, , pH 6.08
  - H2O , pH 8.01
  - 0.5M NH4OH aq. , pH 10.34
- Sample Application: The pre-treated plasma was loaded on to the plate, a pulse of vacuum applied to initiate flow and the samples left to absorb for 5 minutes.
- Elution: Addition of 1 mL of various water immiscible extraction solvents. The extraction solvents tested were:
  - 98:2 (v/v) hexane/3-methyl-1-butanol,
  - 90:10 (v/v) DCM/IPA,
  - EtOAc,
  - DCM
  - MTBE.
- Post Extraction: The eluate was evaporated to dryness and reconstituted in 0.5 mL of 70:30 (v/v) H2O/MeOH prior to analysis.

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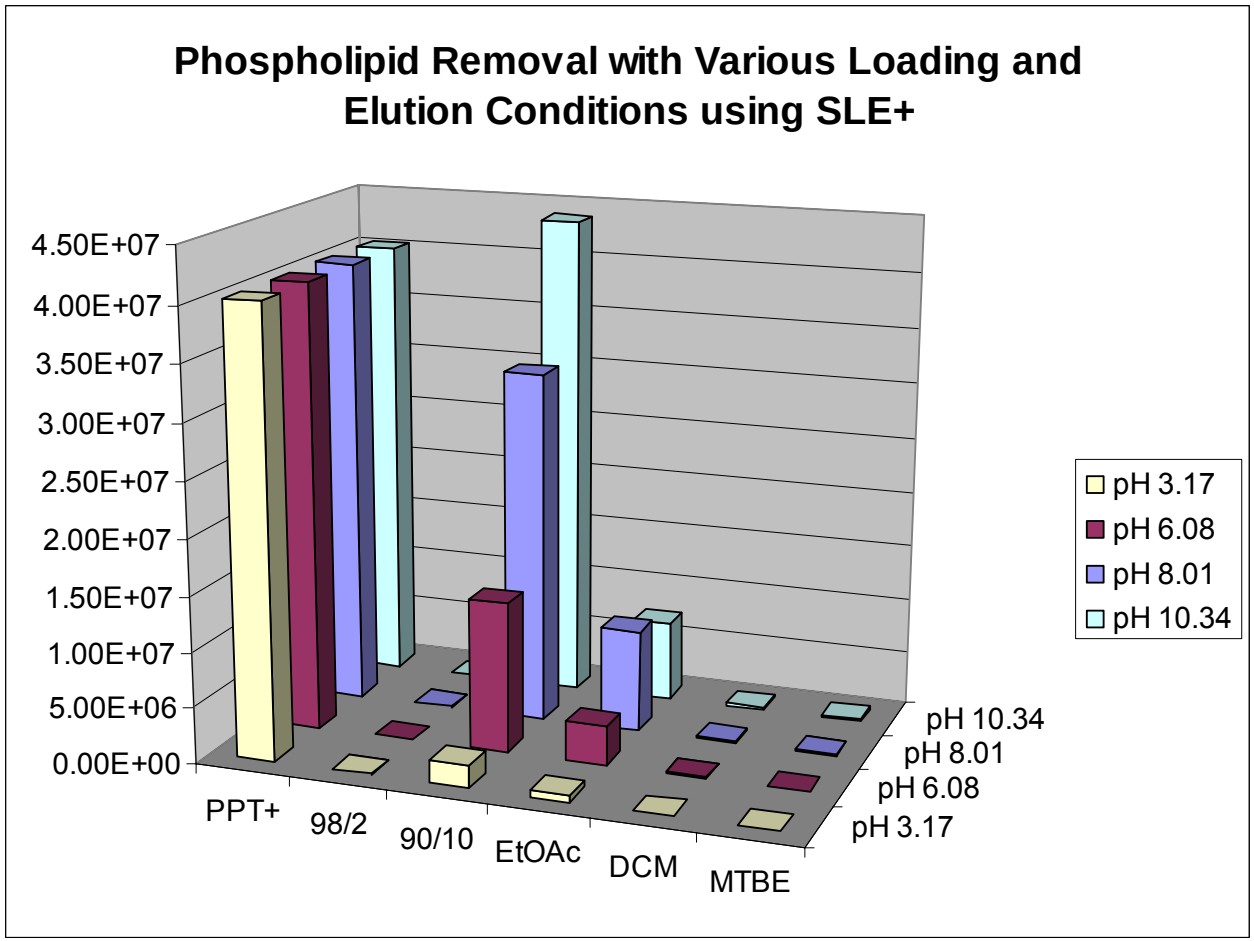
Effectiveness of Phospholipid Removal



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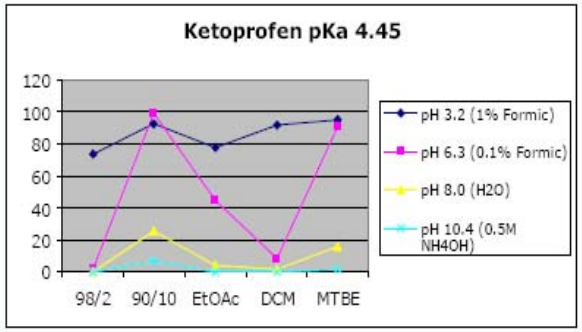
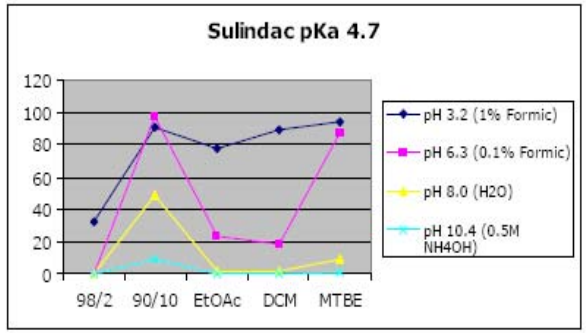
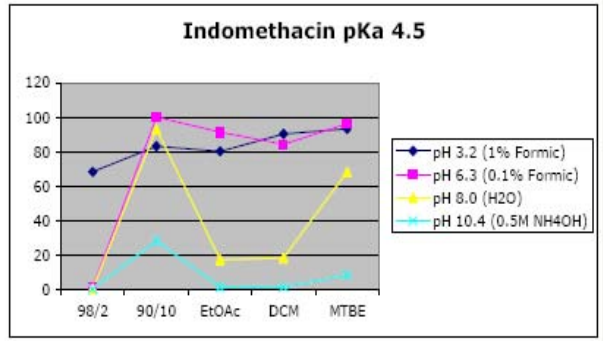
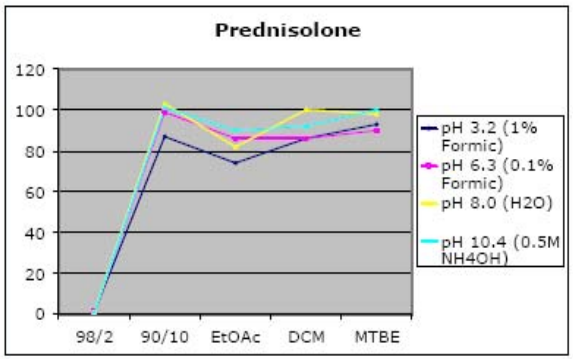
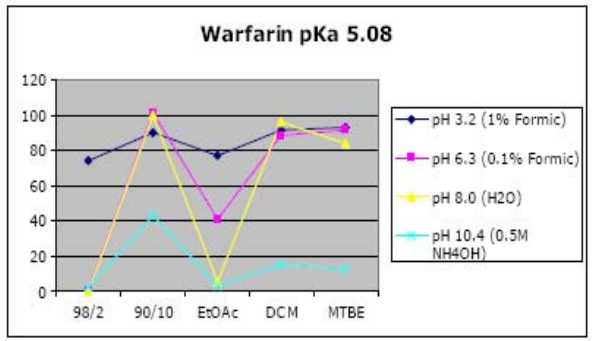
Acidic, Neutral and Basic Compounds

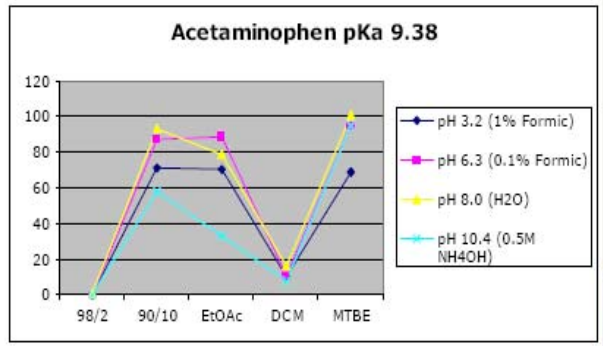


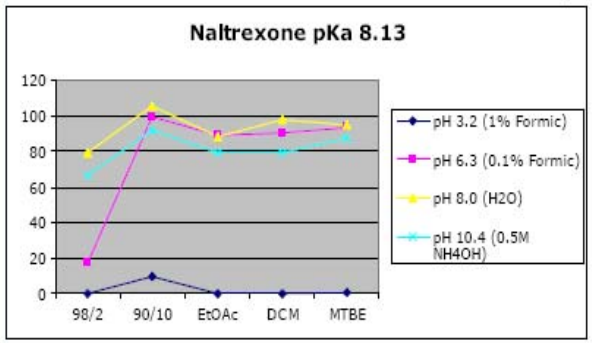


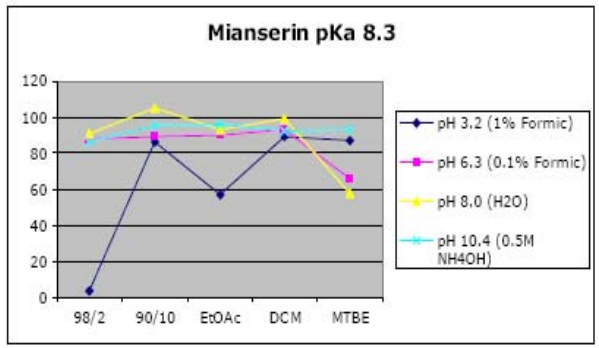












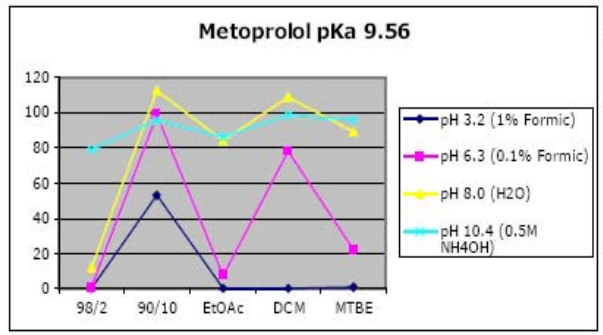


Figure 1. *Comparison of loading pH and elution solvent on analyte recoveries.* 98:2 (v/v) hexane/3-methyl-1-butanol,

Results

- For the acidic and basic analytes the best recoveries were seen when the analytes were in their neutral form, or partially ionized.
- Good recoveries were still possible when the analytes were fully ionized but only with the more polar extraction solvents.
- At pH conditions remote from the pKa values, the polarity of the extraction solvent was not sufficient to elute the analytes.
- The neutral analyte, Prednisolone, showed similar recoveries for each of the extraction solvents at the various pH conditions.

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SLE+ Procedure

1. Dispense pre-buffered sample (200 µL)
2. Apply vacuum (-15”Hg / -0.5 bar) for 2-10 seconds to initiate loading.
3. Wait 5 minutes for sample to completely absorb.
4. Apply extraction solvent (1 x 1 mL). A vacuum pulse is not usually necessary, but can be used in those cases that need it.
5. Allow solvent to flow for 5 minutes under gravity.
6. Apply vacuum (-15”Hg / -0.5 bar) for 2 minutes to complete elution.
7. Evaporate to dryness. Reconstitute in mobile phase prior to analysis.

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SLE+ Format

Be sure to use mat when processing partial plates to ensure vacuum applied is effective.

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