Simultaneous determination of UV-filters and estrogens in aquatic invertebrates by modified QuEChERS extraction and liquid chromatography tandem mass spectrometry

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Estrogens and UV-filters have attracted increased attention as contaminants of emerging concern (CECs)

Estrogens:

- Cause endocrine disruption (*e.g.*, feminization of male fish) [1,2]
- Bioaccumulate in aquatic organisms [3,4]

UV-filters:

- Bioaccumulate in aquatic organisms [2,5]
- Demonstrate estrogenic activity [3]
- Exhibit toxic impacts on coral reefs [6]

1. C. Schmitt *et al*, 2008; 2. R. Cespedes, 2004; 3. A.M. Al-Ansari et al, 2013; 4. B. Huerta *et al*, 2015; 5. S. Morrison *et al*, 2016; 6. C. Downs *et al*, 2014.

Motivation for simultaneous determination of estrogens and UV-filters in invertebrate tissue

- LC-MS based methods for simultaneous determination of multiple estrogens and UV-filters are not available;
- Protocols for effective co-extraction of estrogens and UV-filters from tissue samples are scarce; and,
- Invertebrates, which have a limited amount of tissue, play important ecological roles have not been rigorously studied.

Objectives of this talk

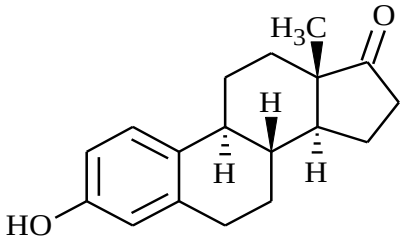
1. Analyze estrogens and UV-filters simultaneously with one LC-MS/MS method;

2. Develop efficient extraction (a) and cleanup (b) strategies to extract three estrogens and five UV-filters from tissue samples; and,

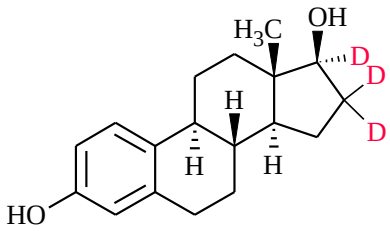
3. Examine estrogen and UV-filter concentrations in aquatic and marine invertebrates (*i.e.*, *Orconectes virilis* and *Crassostrea virginica*) collected in Maryland.

Part I: Simultaneous determination of estrogens and UV-filters with LC-MS/MS

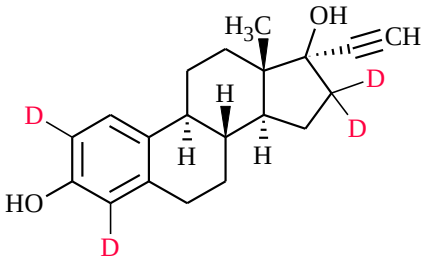
Chemical structures of analytes and internal standards



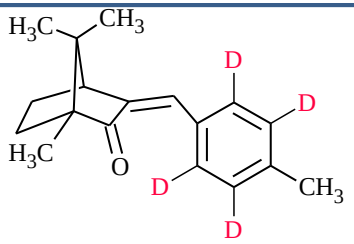
Estrone (E1, E2-d_3)



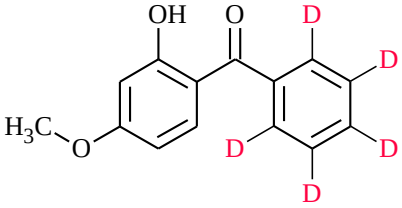
Estradiol (E2, E2-d_3)



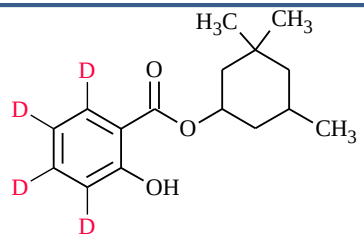
Ethinyl estradiol
(EE2, EE2-d_4)



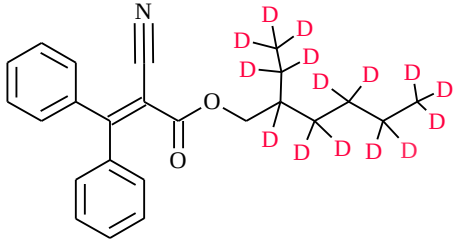
4-methylbenzylidene camphor
(4-MBC, 4-MBC-d_4)



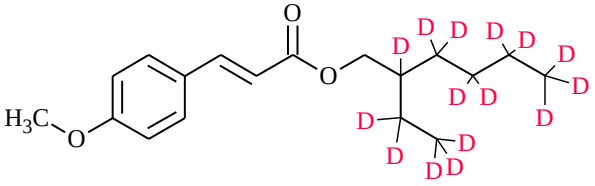
Benzophenone-3
(BP-3, BP-3-d_5)



Homosalate (HMS, HMS-d_4)

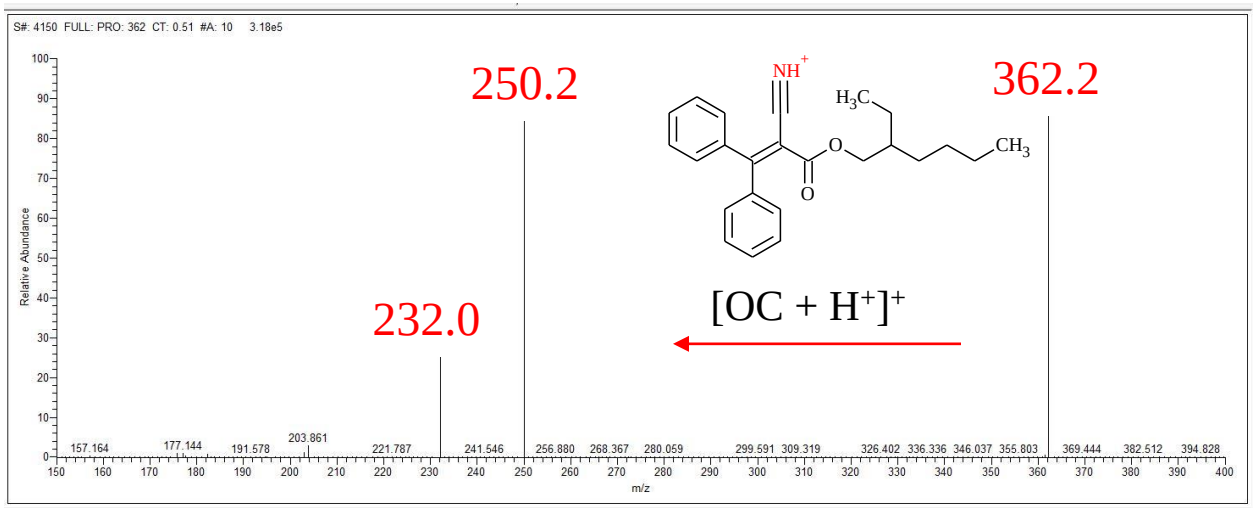


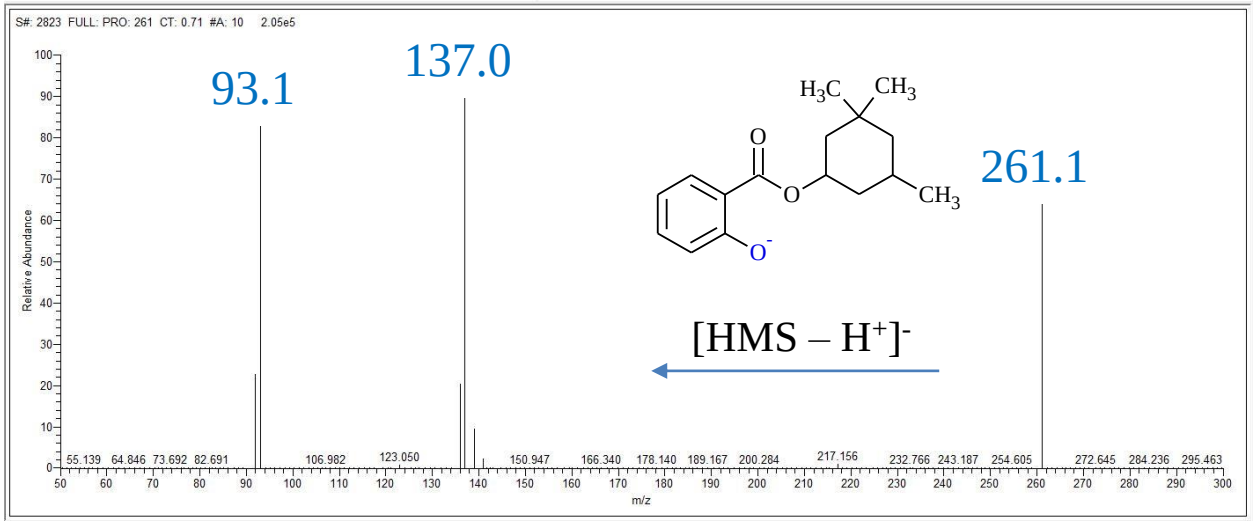
Octocrylene (OC, OC-d_{15})



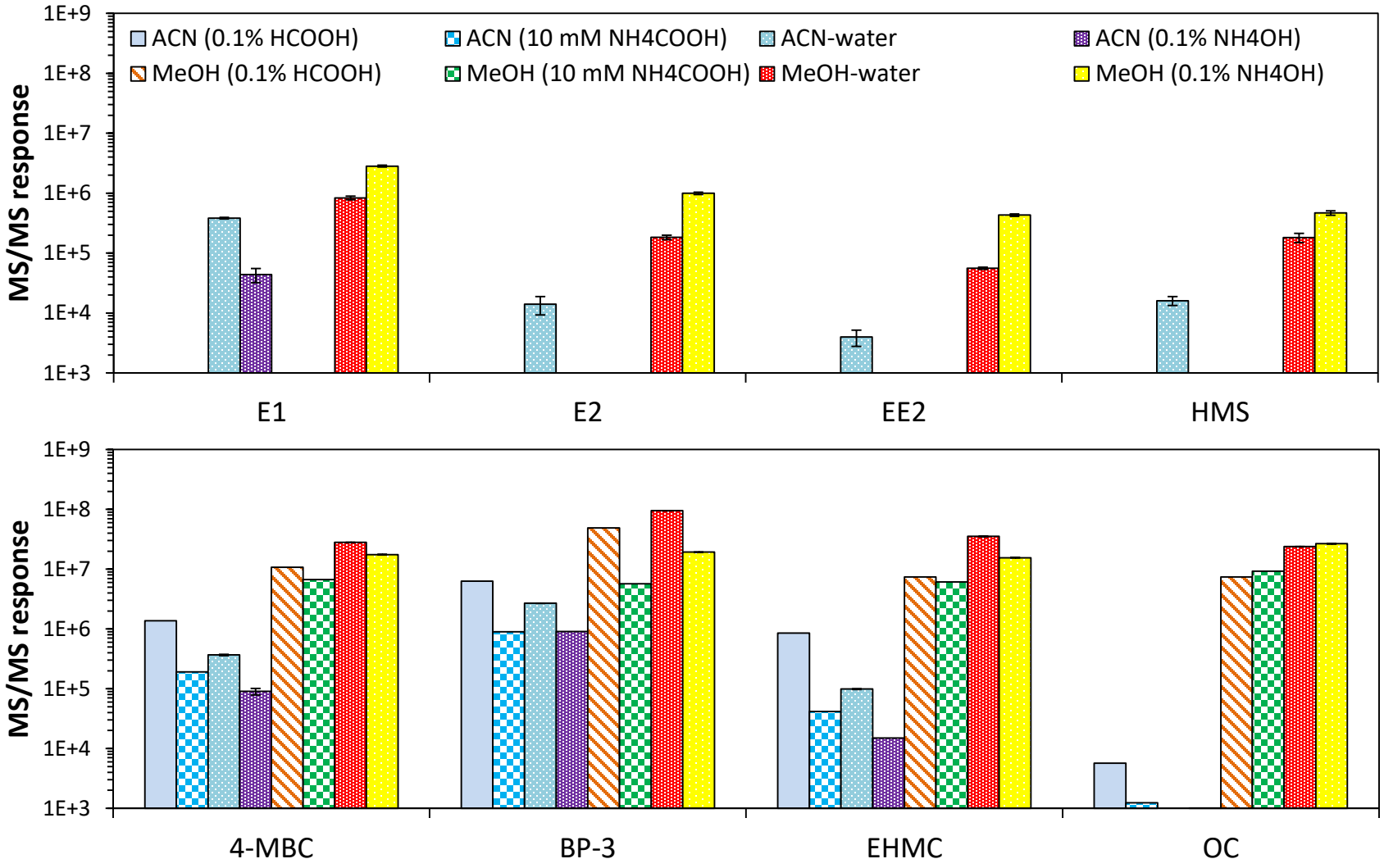
Ethylhexylmethoxycinnamate
(EHMC, EHMC-d_{15})

Positive and Negative ESI-MS/MS fragmentations for OC and HMS



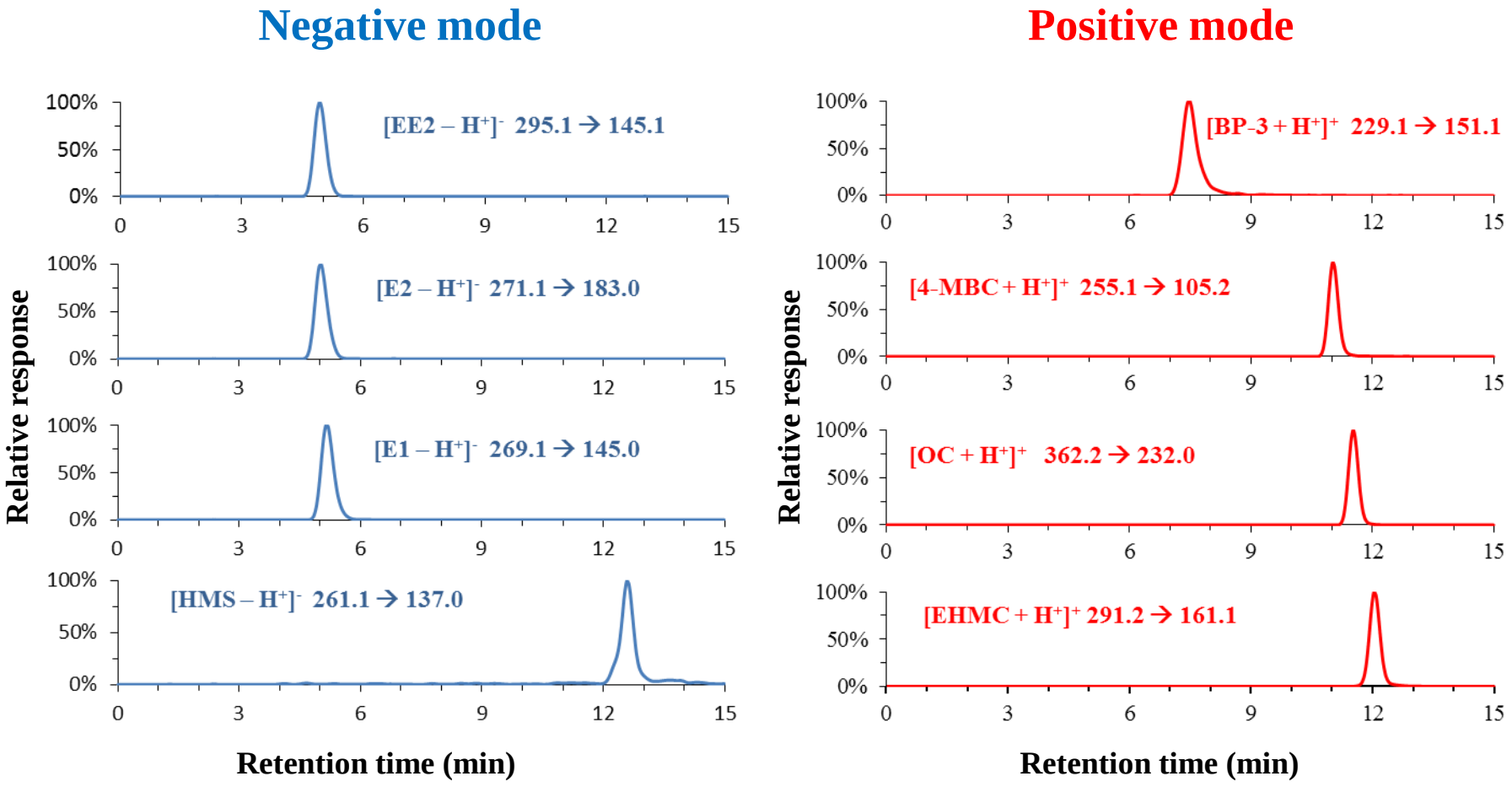


Wrong-way-round ionization behavior of select UV-filters



Analytes were prepared at 10 µg/L in water; each sample was injected five times.

LC-MS/MS workload and sensitivity were improved with wrong-way-round ionization

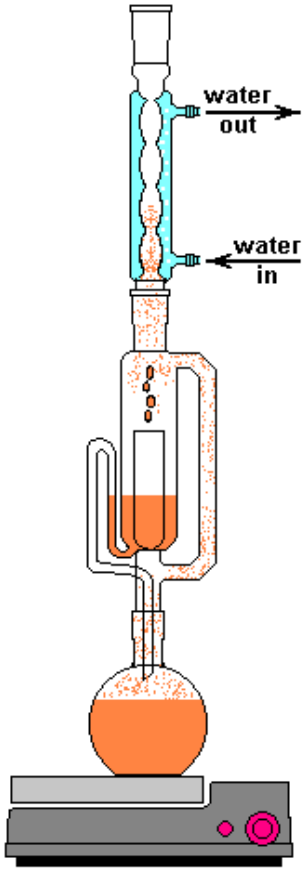


Analytes were prepared at 10 µg/L. A Waters Xbridge BEH C18 column (2.1×150 mm, 2.5 µm) was used for separation. The elution gradient employed (A) water with 0.1% NH_4OH (pH 10.5) and (B) MeOH with 0.1% NH_4OH at a flow rate of 0.2 mL/min.

Part II (a): Extraction of estrogens and UV-filters from tissue samples

Conventional techniques involve “one-step” extraction

Soxhlet extraction



Accelerated solvent extraction (ASE)

Sonication/ultrasound assisted extraction

Ref: https://commons/d/d2/Soxhlet_mechanism.gif; http://media.americanlaboratory.com/m/20/article/743-Fig1.jpg

The QuEChERS (Quick, Easy, Cheap, Efficient, Rugged, and Safe) strategy provides "two-step" extraction

We employed a modified QuEChERS extraction as indicated below:

50 mg freeze-dried tissue samples

↓

5 mL DI + 5 mL acetonitrile — **Extract with 50 % ACN**

↓

2.5 g $MgSO_4$ + 1 g NaCl — **Extract with ≈ 92 % ACN**

↓

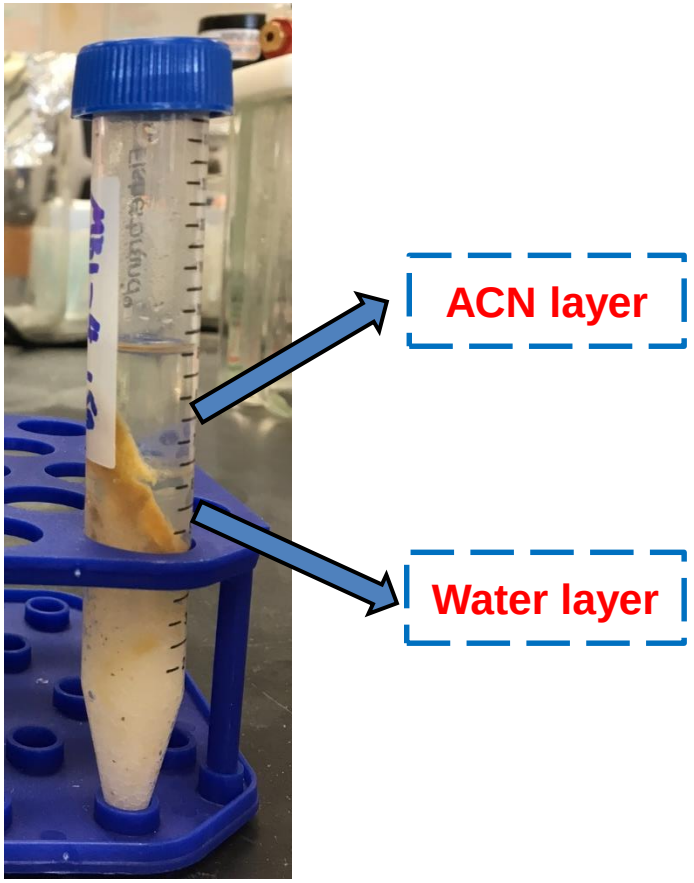
Aliquot of 2.5 mL up layer extract

↓

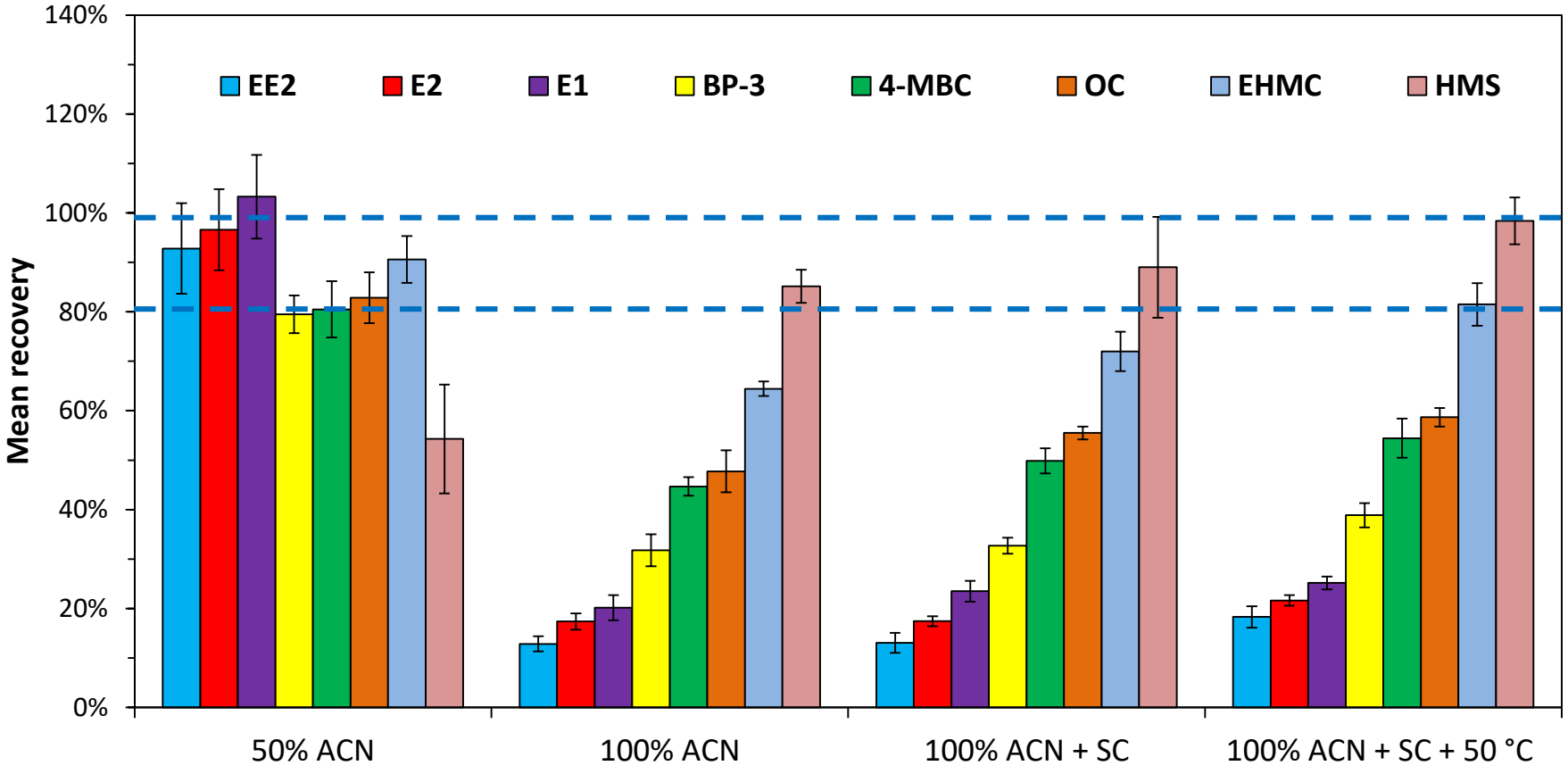
Cleanup with dispersive-SPE

↓

Instrument analysis

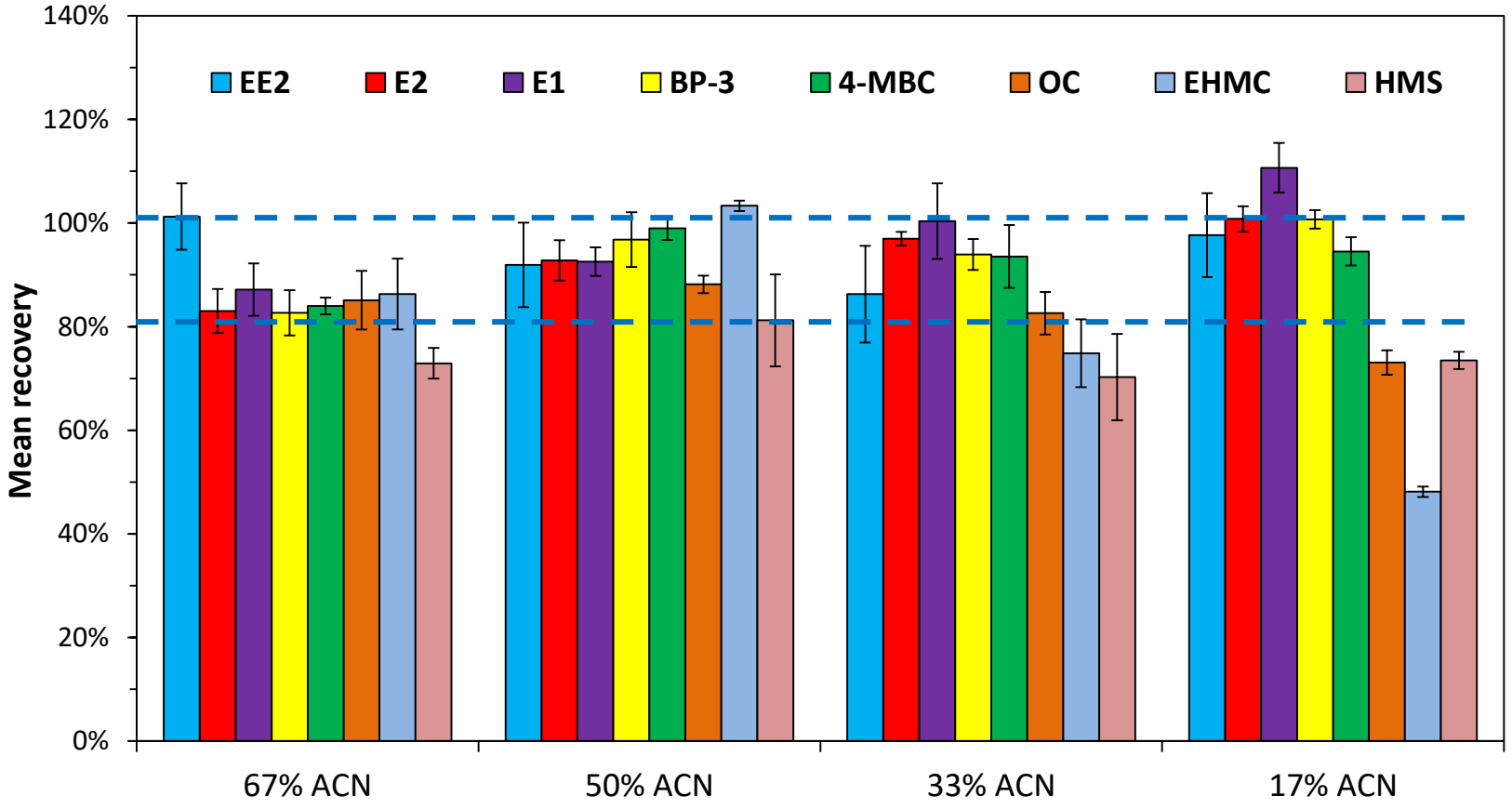


Recovery of estrogens and UV-filters from tissue with ACN extraction



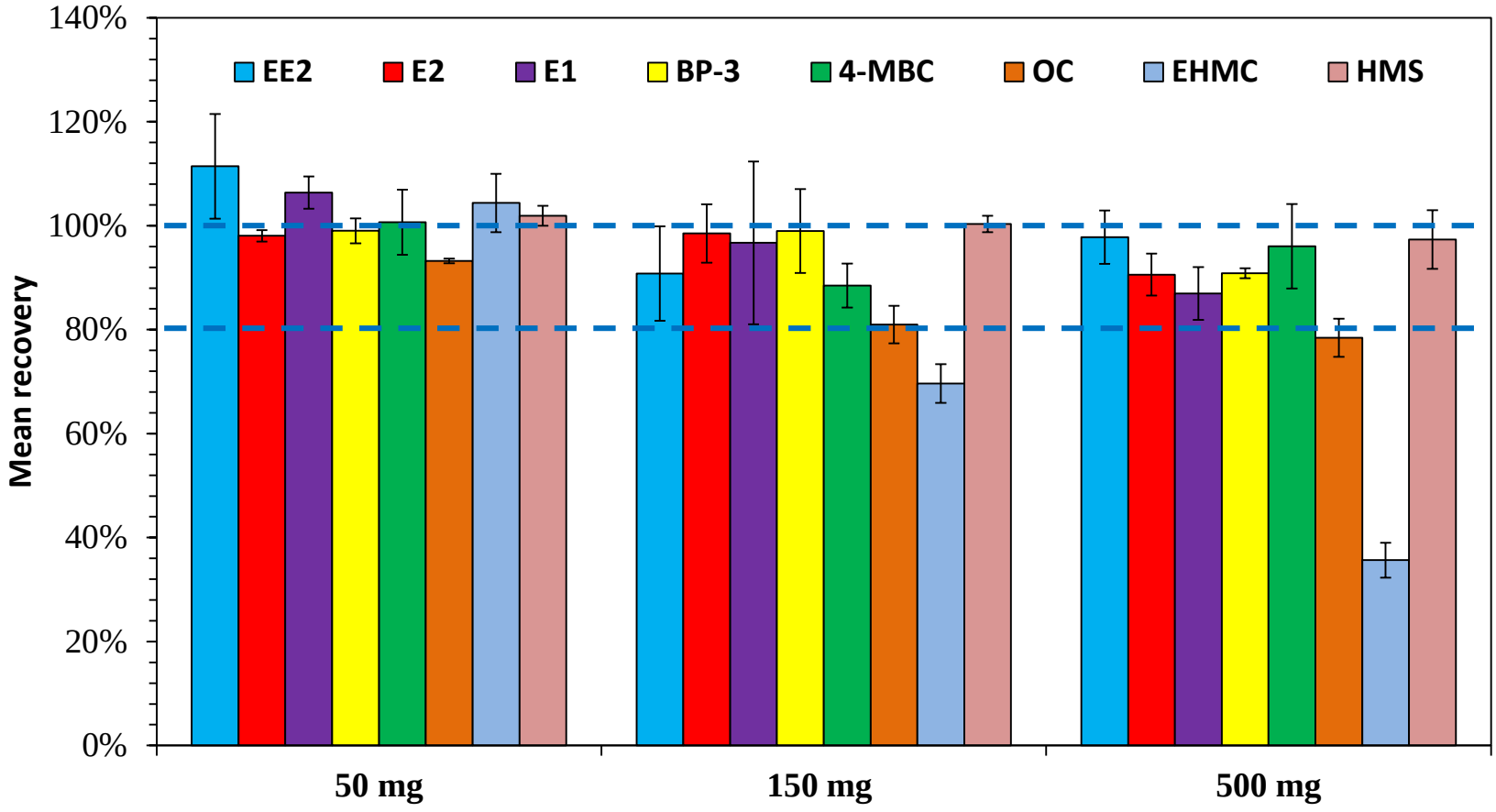
Red swamp crayfish tissue was used for method development; 100 ng of each analyte was spiked into 50 mg freeze-dried tissue mass overnight before extraction with 5 mL solvent; extraction was conducted in triplicate; SC, sonication.

Recovery of estrogens and UV-filters in tissue with QuEChERS extraction at different initial ACN content



100 ng of each analyte was spiked into 50 mg freeze-dried tissue mass overnight before extraction with 10 mL solvent; extraction was conducted in triplicate.

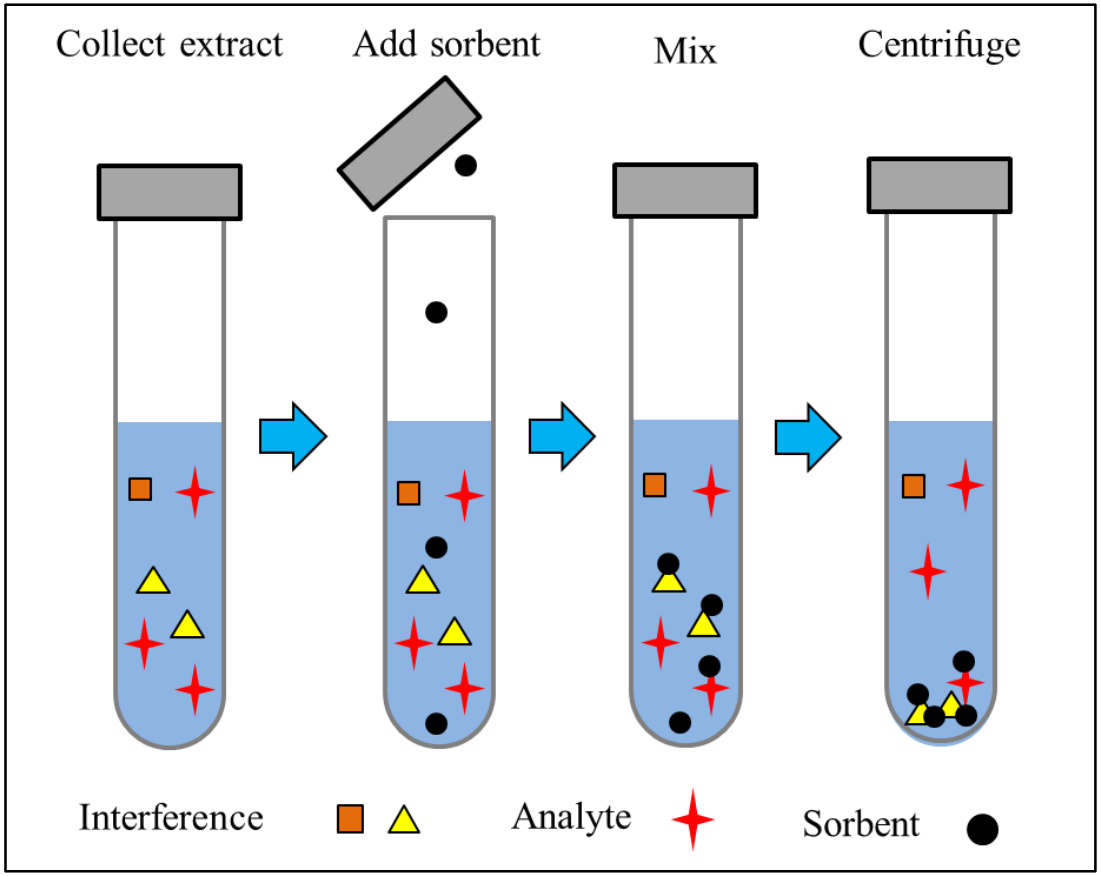
Estrogens and UV-filters were effectively recovered using the modified QuEChERS extraction strategy



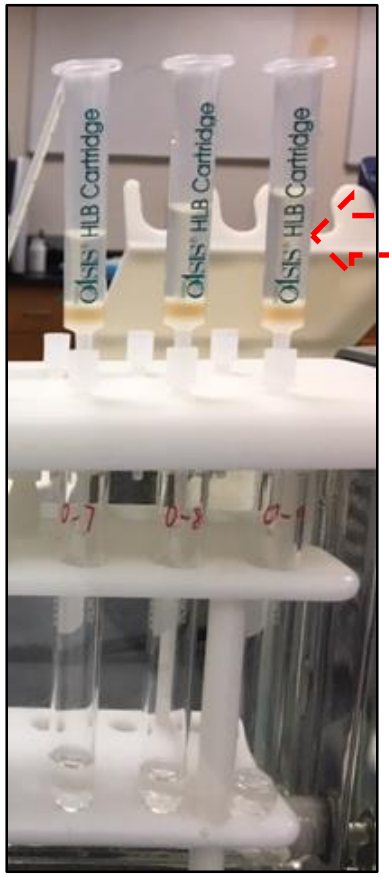
100 ng of each analyte was spiked into 50, 150, and 500 mg freeze-dried tissue mass overnight before extracteionwith 5 mL ACN and 5 mL water; extraction was conducted in triplicate.

Part II (b): Cleanup with a novel reverse-solid-phase extraction (reverse-SPE) process

The dispersive-SPE idea was adopted and further developed as reverse-SPE

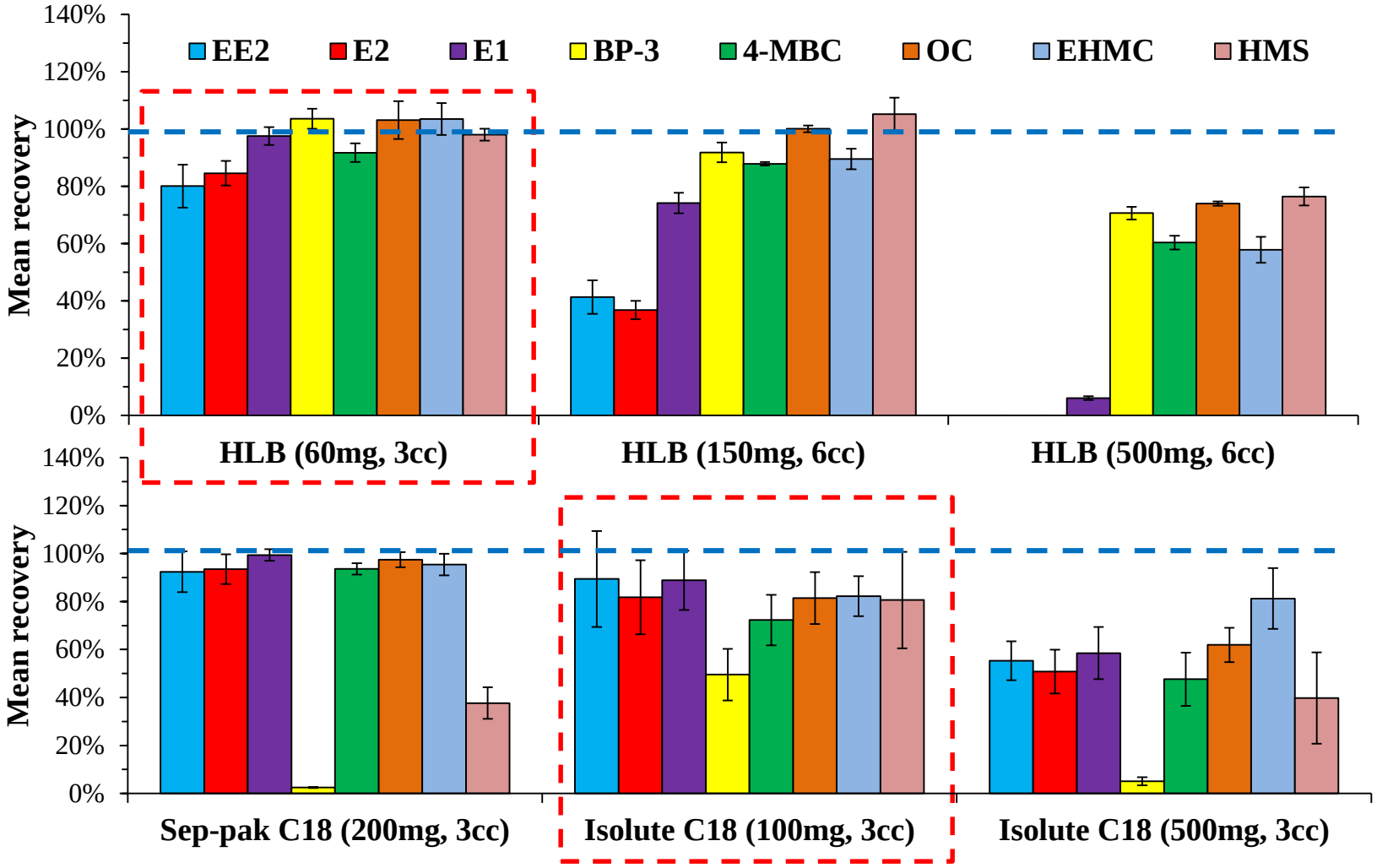


dispersive-SPE cleanup protocol



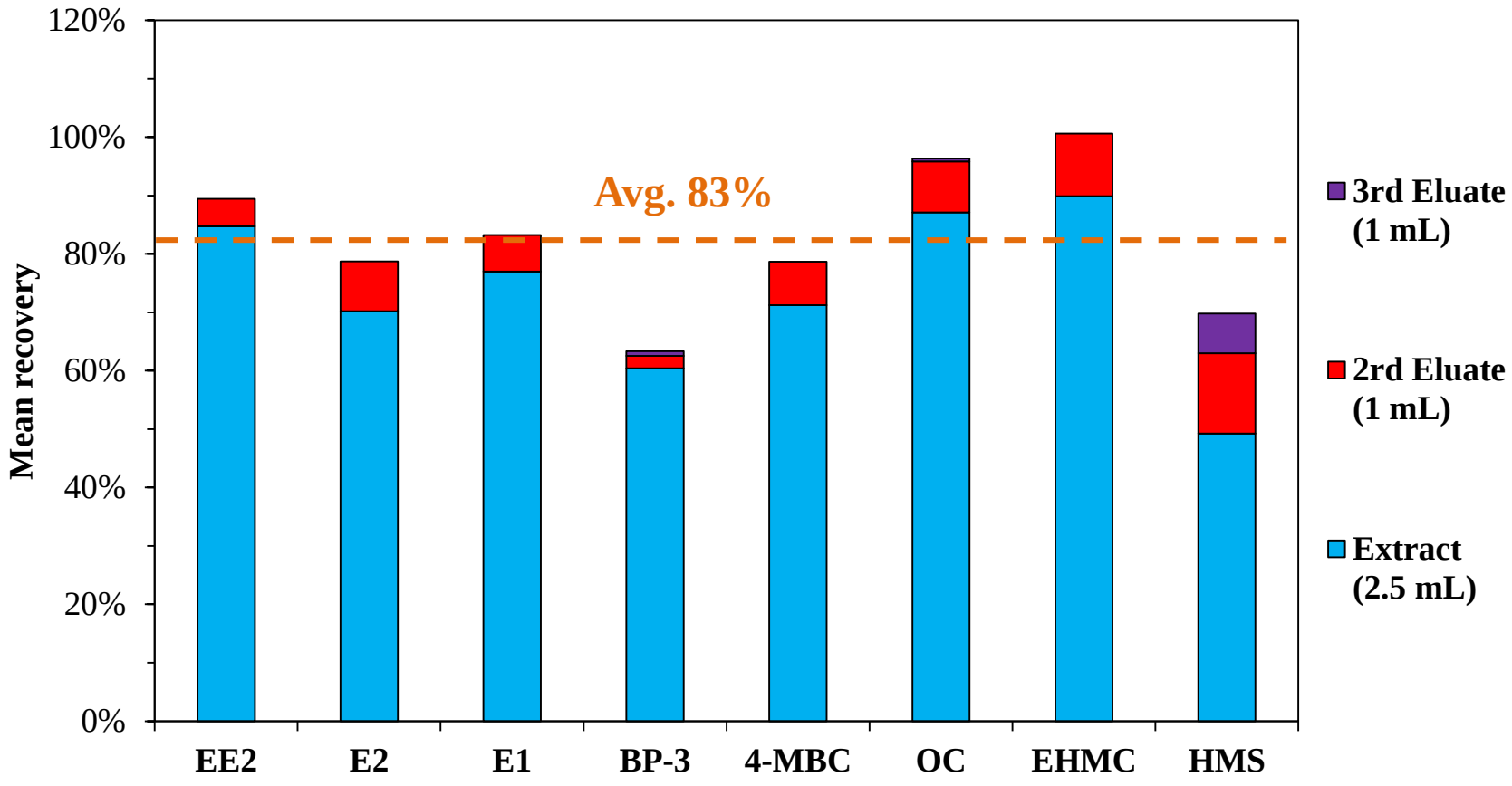
reverse-SPE strategy

Recovery of estrogens and UV-filters in 5 mL ACN through different cartridges during cleanup



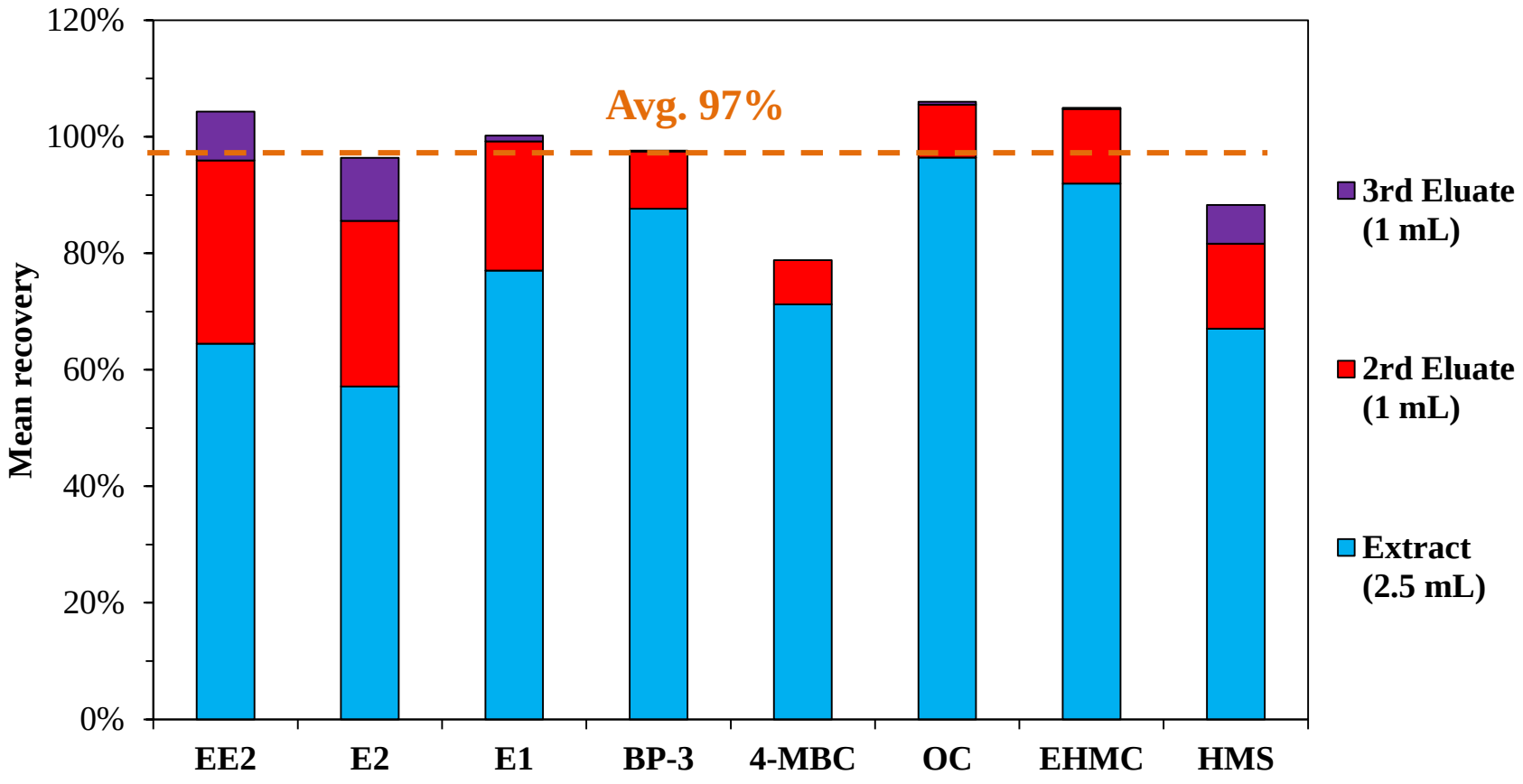
The concentration was 10 µg/L in 5 mL ACN for each target compound; flow rate was by gravity.

Isolute C18 (100 mg, 3 cc) cartridge provides acceptable recovery of estrogens and UV-filters



The spiked concentration was 10 µg/L in the 2.5 mL extract for each target; flow rate was by gravity.

HLB (60 mg, 3 cc) cartridge provides better recovery for estrogens and UV-filters



The spiked concentration was 10 µg/L in the 2.5 mL extract for each target compound; flow rate was by gravity.

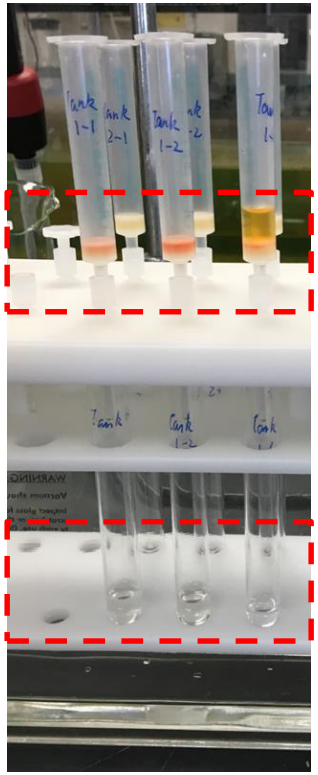
Reverse-SPE with HLB decreased matrix effects (ME)

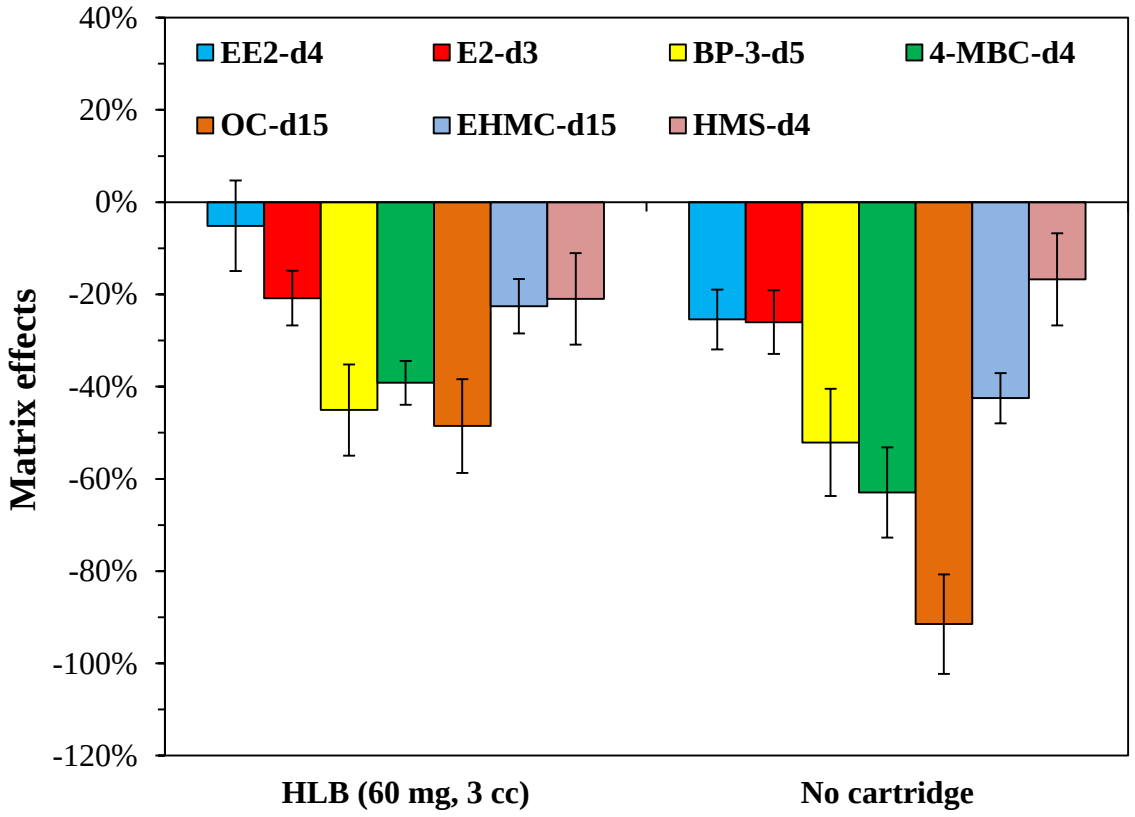
$$ME = \frac{R_{SO} - R_O}{R_S} - 1$$

R_{so} - response of the spiked analyte in the sample extract;
R_o - response of the unspiked sample extract;
R_s - response of the spiked analyte in the mobile phase.

Extract after salting out

Eluate for N_2 evaporation





The seven internal standards were spiked at 5 µg/L in the reconstitution solution.

Part III: Occurrence of estrogens and UV-filters in the aquatic and marine environment in Maryland

Crayfish and oyster sampling strategies

Collected from a watershed

Used electric shock

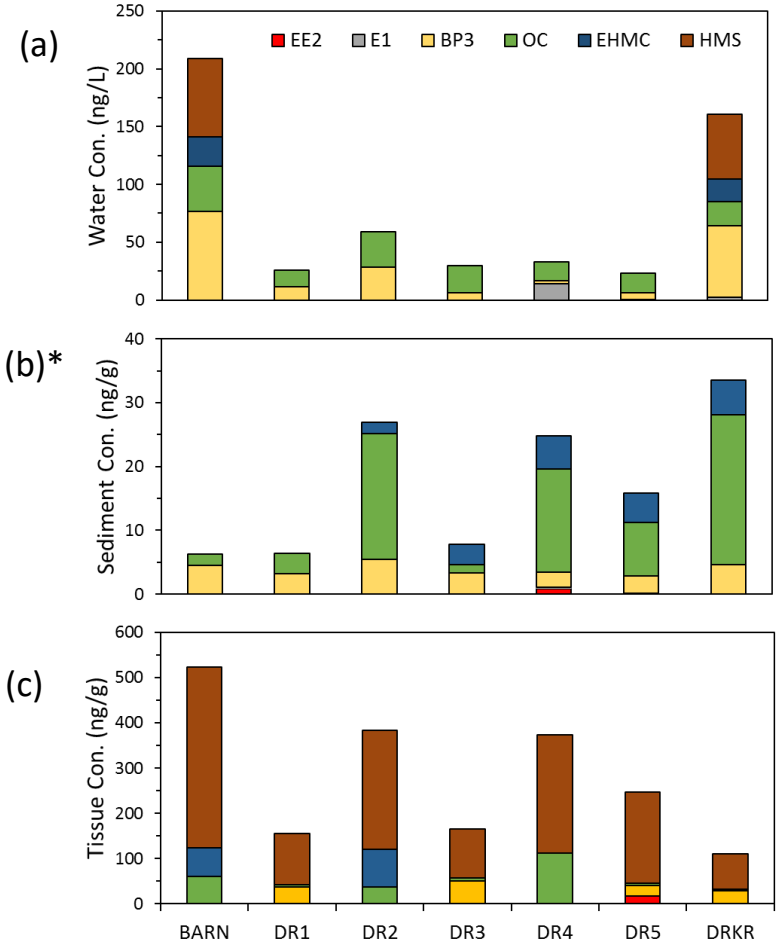
Randomly picked 20 crayfish

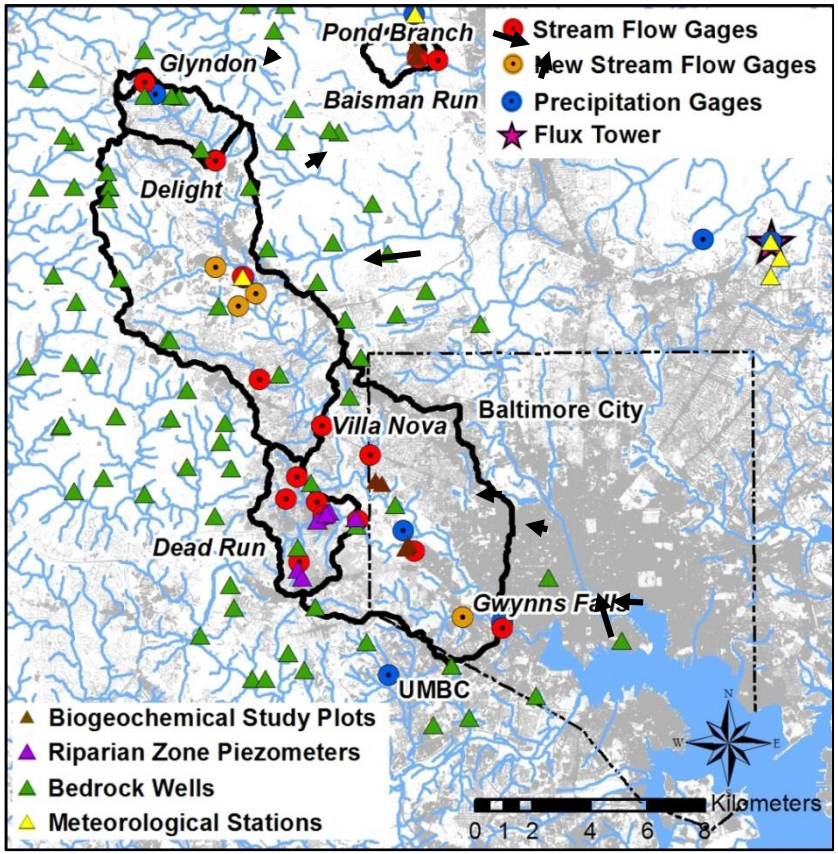
Collected from the Chesapeake bay

Used hydraulic dredge

Randomly picked 3 oysters

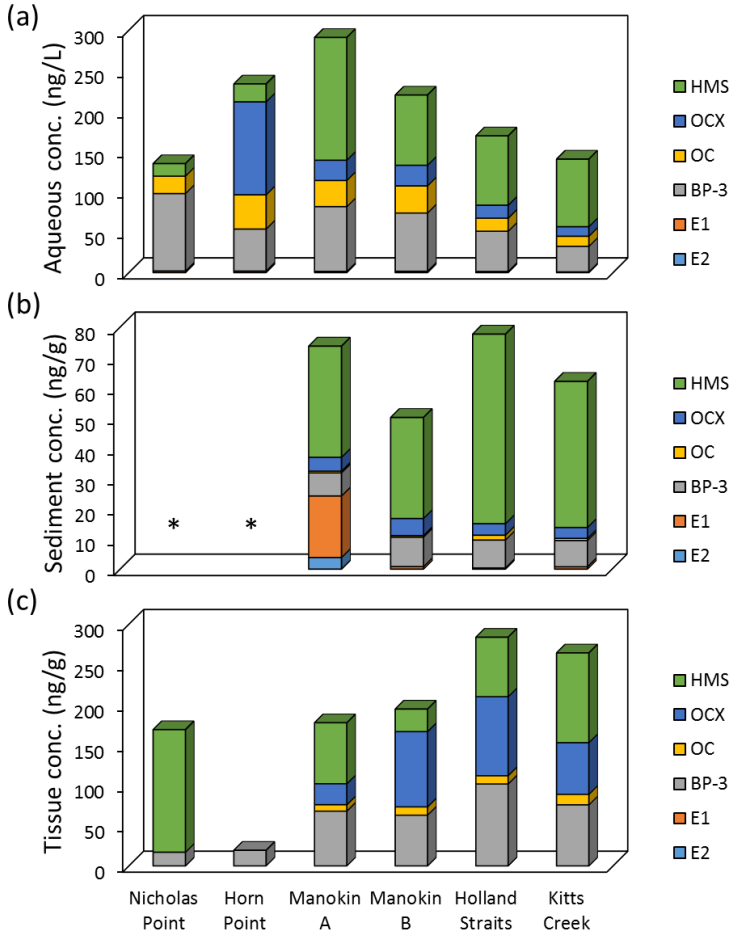
Detection of estrogens and UV-filters in (a) stream water, (b) sediment, and (c) crayfish from the Gwynns Falls watershed (Baltimore, MD)





BARN, Baisman Run; DR1-5, Dead Run Sites 1-5; DRKR, Dead Run at Franklintown; * HMS was not included for the analysis of sediment samples.

Detection of estrogens and UV-filters in (a) seawater, (b) sediment, and (c) oysters from the Chesapeake Bay



Conclusions

- Estrogens and UV-filters were simultaneously analyzed in LC-MS/MS using wrong-way-round ionization behavior;
- Low detection limits (*i.e.*, 0.2 – 2.0 ng/g) were achieved by processing a small sample size (*i.e.*, 50 mg) with a modified QuEChERS protocol followed by a novel reverse-SPE cleanup;
- All eight target analytes were detected at least once in the tissue samples, with the highest concentration being 399 ng/g homosalate in *O. virilis*; and,
- Given the high detection frequency of these CECs, it is important to investigate their potential impacts on invertebrates.

Acknowledgements

- CBEE colleagues, UMBC

  Kiranmayi Mangalgiri, Utsav Shashvatt, John Kemper, and Elvis Andino Nolasco for their help in crayfish sample collection; Ethan Hain for his help in sample preparation and analysis.

- Department of Natural Resources, Maryland

  Mitchell Tarnowski and his colleagues for their help in oyster sample collection.

- US Department of Agriculture Forest Service

  USDA Forest Service project #7403

Thank you!

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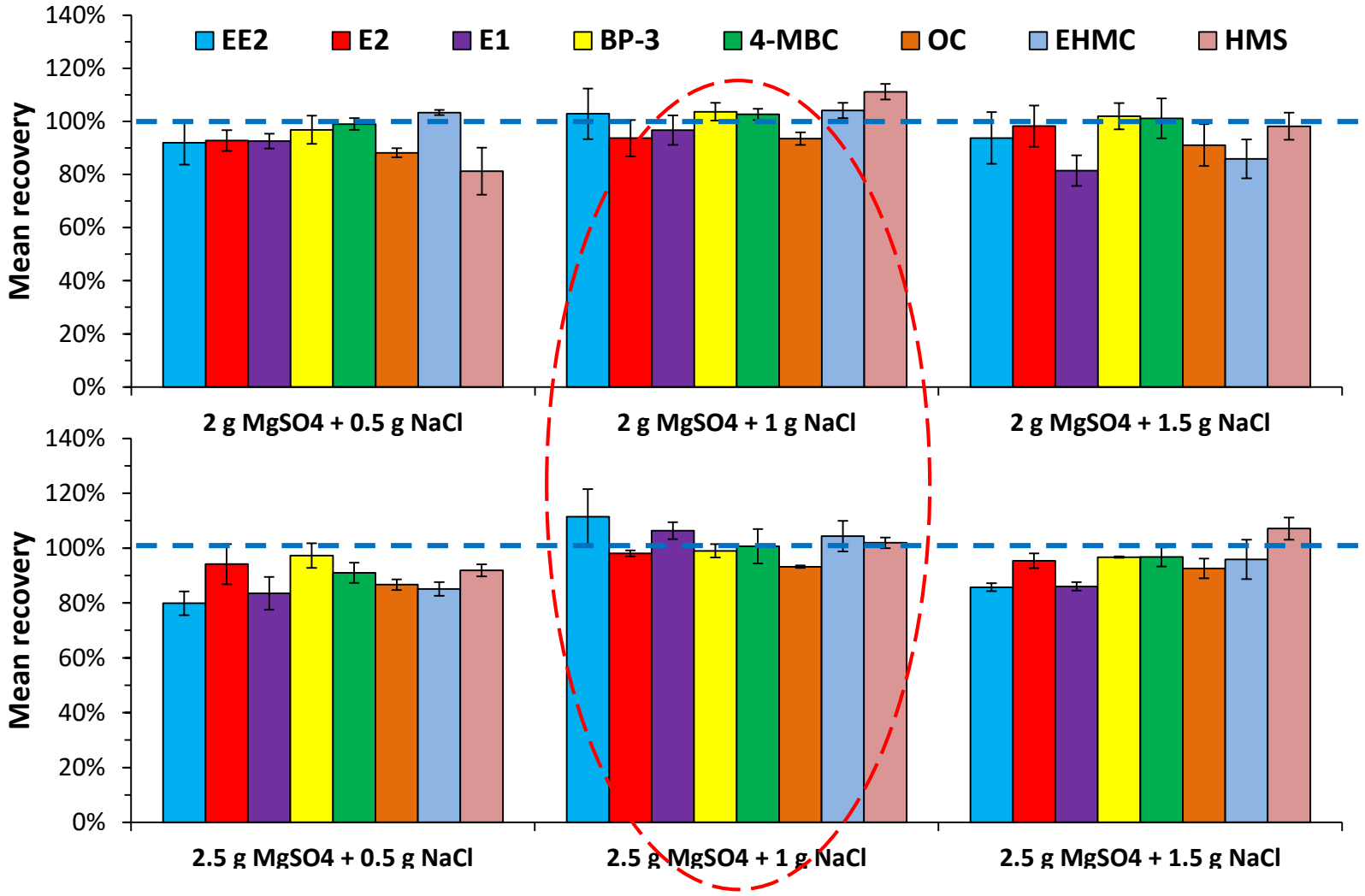
Simultaneous determination of UV-filters and estrogens in aquatic invertebrates by modified quick, easy, cheap, effective, rugged, and safe extraction and liquid chromatography tandem mass spectrometry

Ke He[a], Anne Timm[b], Lee Blaney[a,*]

Supplementary slides

Impact of salt conditions on recovery of estrogens and UV-filters



100 ng of each analyte was spiked into 50 mg freeze-dried tissue mass overnight before extraction with 5 mL ACN and 5 mL water; extraction was conducted in triplicate.

Recovery of UV-filters and estrogens in water, sediment, and tissue samples.