Quantification of **BODIPY**-conjugated 17β-estradiol and estrone by **HPLC-PDA** following solid phase extraction versus traditional **GC-MS**

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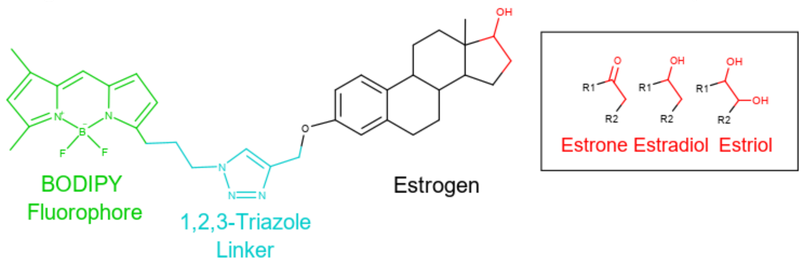
Introduction

Estrogens—including estrone (E1), 17β-estradiol (E2), estriol (E3), and their synthetic derivatives—have been identified as emerging contaminants which disrupt endocrine and reproductive functions in fish[1,2]. Currently, the most common platform for trace analysis of estrogens, GC-MS, can be prohibitively expensive to maintain and operate[3]. Therefore, there is incentive to develop sensitive analytical methods which are accessible to facilities not equipped with mass spectrometers.

In the present work, we developed an HPLC-diode array detection method for quantifying E1 and E2. BODIPY azide was previously coupled to E1 and E2 at -OH of C3 via click chemistry, forming a 1,2,3-triazole linker between the structures (work done by R. Marquez et al)[4].

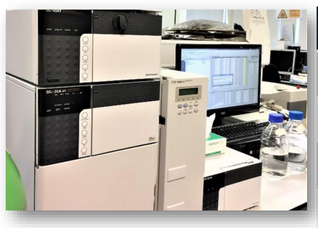


Experimental Methods

We evaluated the HPLC and GCMS methods using unextracted standards, adhering to both the Eurachem Guidelines of Analytical Method Validation and the EU Method Validation of Estrone and 17β-Estradiol[5,6]. The non-tagged HPLC[7], GCMS[8] and SPE[6] methods used for comparison were adapted from the literature.

Shimadzu Prominence HPLC-PDA	
Mobile Phase A:B	H_2O:CH_3CN, 0.5 mL/min
Gradient	40%-100%B (+5%B/min)
Column	Purospher RP-18 (35°C) 4.6×150mm, 5µm
Acquisition	Non-tagged: 230 nm; BDP-tagged: 503 nm

Agilent 6890/5975C GC-MSD	
Derivatization	BSTFA:ACN, 100min 85°C
EI Source	70eV, 230°C
Column	DB-5 30m×0.25mm×0.25µm
Injection	Splitless
Acquisition	SIM: TMS-E1 @ 342 *m/z*; bisTMS-E2 @ 416 *m/z*



Solid Phase Extraction	
Cartridge	Oasis HLB, 3cc, 60mg
Condition	3 mL EtOAc, CH_3OH, H_2O
Sample Volume	15 mL (Milli-Q H_2O)
Elution Volume	1 mL EtOAc
Reconstitution	0.15 mL CH_3CN

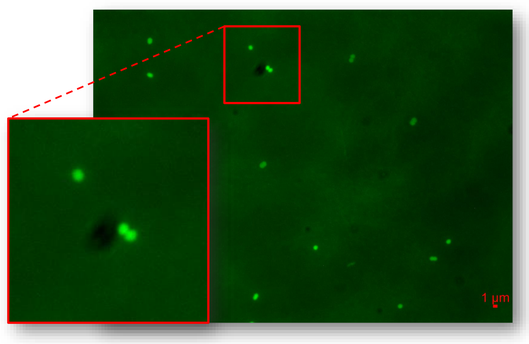
Conclusions & Future Work

In the present work, we have demonstrated that BODIPY-conjugated estrogens have greater analytical performance than non-tagged estrogens analyzed by HPLC and TMS-derivatized estrogens analyzed by GCMS.

We hope to incorporate the Cu(I)-catalysed 1,3-dipolar cycloaddition click reaction into a derivatization protocol for trace analysis of estrogens.

Potential Applications

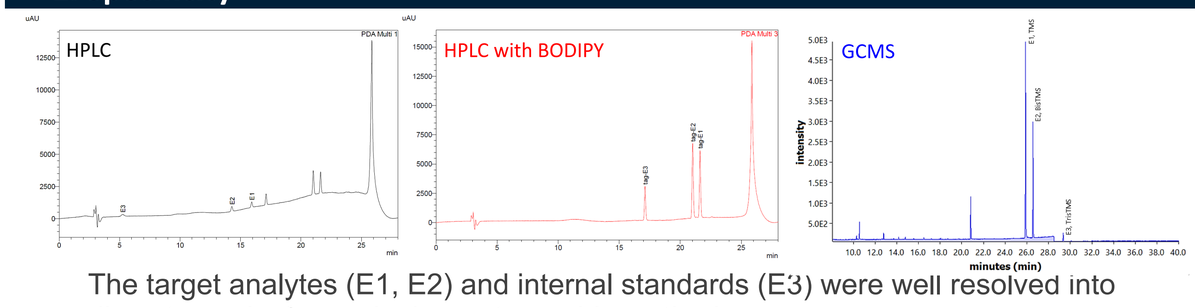
- Pre-Experimental Derivatization Applications
  - BODIPY is widely used in biological imaging, and therefore, could be used for biological degradation studies, as shown in the fluorescent microscope image.
  - This approach is useful for studies not dependent on physical properties of the analyte (e.g. size, polarity).
- Post-Experimental Derivatization Applications
  - Size exclusion, nanofiltration and sorption removal studies; studies which are dependent on physical properties of the analyte.



Fluorescent image of *Nitrosomonas europaea* NCIMB11850. Fluorescent dye is BODIPY-E2.

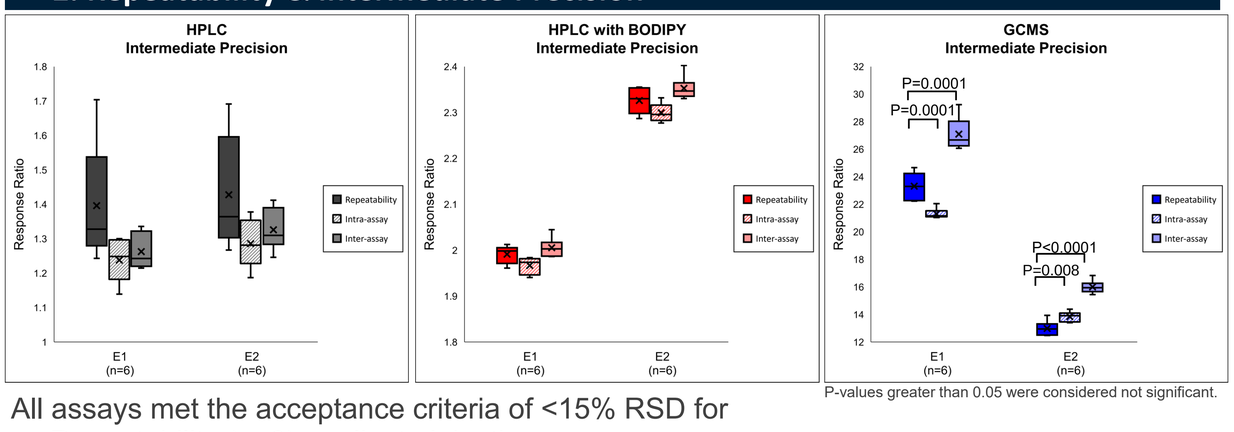
Results

1. Specificity



The target analytes (E1, E2) and internal standards (E3) were well resolved into discrete peaks for each chromatographic method.

2. Repeatability & Intermediate Precision



P-values greater than 0.05 were considered not significant.

All assays met the acceptance criteria of <15% RSD for

- Repeatability (n=6): replicate injections,
- Intra-Assay Precision (n=12): replicate injections at the start and end of a batch, and
- Inter-Assay Precision (n=12): replicate injections at the start of a batch on two non-consecutive days.

%RSD	HPLC			HPLC with BODIPY			GCMS		
	Repeat.	Intra-Assay	Inter-Assay	Repeat.	Intra-Assay	Inter-Assay	Repeat.	Intra-Assay	Inter-Assay
E1	12.2	10.4	10.5	1.0	1.1	1.0	4.3	5.7	8.9
E2	11.5	10.9	9.4	1.2	1.2	1.2	4.1	4.6	11.3

3. Linearity

R^2 (n=3)	HPLC	HPLC with BODIPY	GCMS
Range	0.22-0.88µM	0.074-0.74µM	0.074-0.74µM
E1	0.9911 ±0.005	0.9989 ±0.001	0.9923 ±0.005
E2	0.9878 ±0.009	0.9990 ±0.001	0.9920 ±0.001

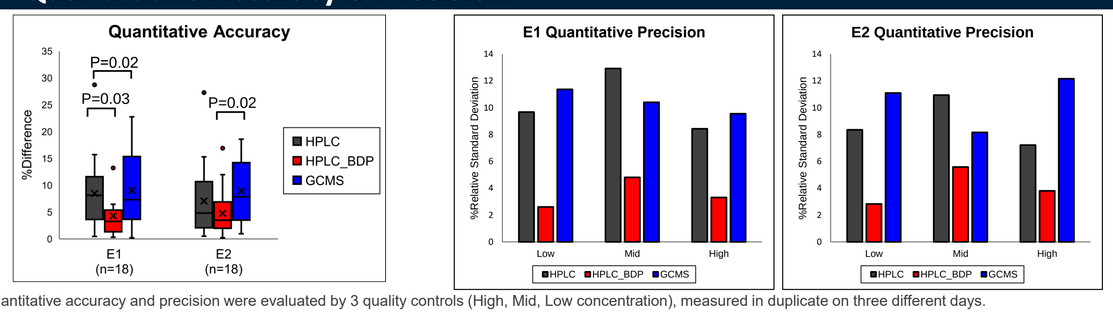
R^2 presented is the average from three 6-point calibration standard curves. Each calibration standard series was prepared fresh on three different days.

4. Limits of Detection

Instrument LOD (n=3)	HPLC	HPLC with BODIPY	GCMS
E1 µM	0.079 ±0.026	0.027 ±0.013	0.072 ±0.030
E2 µM	0.090 ±0.040	0.025 ±0.012	0.077 ±0.006

LOD=3·$S_{y/x}$/slope, averaged from 3 standard curves

5. Quantitative Accuracy & Precision



Quantitative accuracy and precision were evaluated by 3 quality controls (High, Mid, Low concentration), measured in duplicate on three different days.

5. SPE Recovery

Recovery was evaluated by comparing the response of analyte spiked before extraction (n=6) against response of post-extraction spike (n=6), measured in duplicate by HPLC.

In Brief

- BDP-E1 and -E2 were detectable with **greater S/N** than HPLC of non-tagged estrogens.
- HPLC of BDP-E1 and -E2 showed greater **repeatability and quantitative accuracy and precision** than HPLC of non-tagged estrogens and GCMS of TMS-estrogens.
- BDP-E1 and -E2 had a calculated instrument LOD of 0.025 and 0.027µM. This is equivalent to **7 ppb** of estrogen.

References & Acknowledgements

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[1] K. A. Kidd, P. J. Blanchfield, K. H. Mills, V. P. Palace, R. E. Evans, J. M. Lazorchak and R. W. Flick, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, 104, 8897-8901.
[2] A. M. Vajda, L. B. Barber, J. L. Gray, E. M. Lopez, J. D. Woodling and D. O. Norris, *Environ. Sci. Technol.*, 2008, 42, 3407-3414.
[3] J. M. El-Khoury, "Financial considerations for purchasing a mass spectrometer," MSACL, 2018.
[4] A. M. Hansen, A. L. Sewell, R. H. Pedersen, D-L. Long, N. Gadegaard, R. Marquez, *Tetrahedron*, 2013, 69, 8527-8533.
[5] B. Magnusson and U. Örnemark, "The Fitness for Purpose of Analytical Methods: A Laboratory Guide to Method Validation and Related Topics", 2014.
[6] S. Tavazzi, S. Comero, M. Ricci, B. Paracchini, G. Mariani and B. M. Gawlik, "Water Framework Directive Watch List Method Analysis of 17β-estradiol and estrone", 2016, EUR 27970 EN.
[7] Y.-L. Chen, C.-P. Yu, T.-H. Lee, K.-S. Goh, K.-H. Chu, P.-H. Wang, W. Ismail, C.-J. Shih and Y.-R. Chiang, *Cell Chem. Biol.*, 2017 24, 712-724.e7.
[8] J. B. Quintana, J. Carpinteiro, I. Rodríguez, R. A. Lorenzo, A. M. Carro and R. Cela, *J. Chromatogr. A*, 2004, 1024 (1–2), 177–185.