LC-MS as a Tool for Engineers Optimizing Wastewater Treatment

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YARGEAU LABORATORY
Controlling
Contaminants of
Concern

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Is a mass spectrometer part of an engineer's toolbox?

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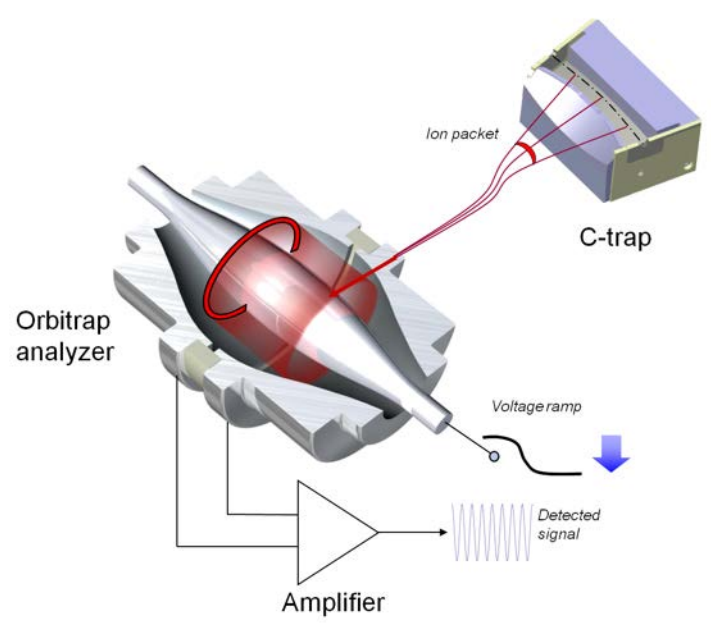

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Controlling Contaminants of Concern

Environmental Assessment

- Presence and fate in surface water
- Impact of wastewater discharges
- Sewage epidemiology

Industrial and municipal wastewater treatment

- Disinfection & Tertiary treatment
- Ozonation, Catalytic ozonation, Photocatalysis

Nature and toxicity of transformation products

- Toxicity testing: Microtox, LuminoTox, YES, YAS, ERα–CALUX
- Identification of TPs

Environmental Assessment

Industrial and municipal wastewater treatment

Nature and toxicity of transformation products

Estrone – Natural hormone

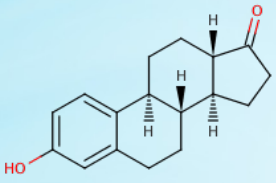
Levofloxacin – Antibiotic

MDMA (Ecstasy) – Illicit drug

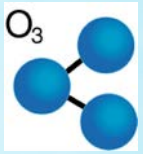
Contaminants of emerging concern (CECs)

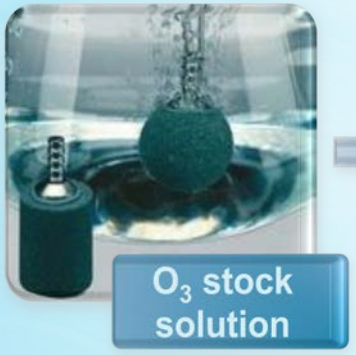
Ozone (O_3) or other AOPs

Experimental approach



Estrone concentration: 0.05 – 2 mg/L
Ozone dose: molar ratios E1:O3 of 1:0 to 1:8
Bioassay: Yeast Estrogen Screening (YES) assay

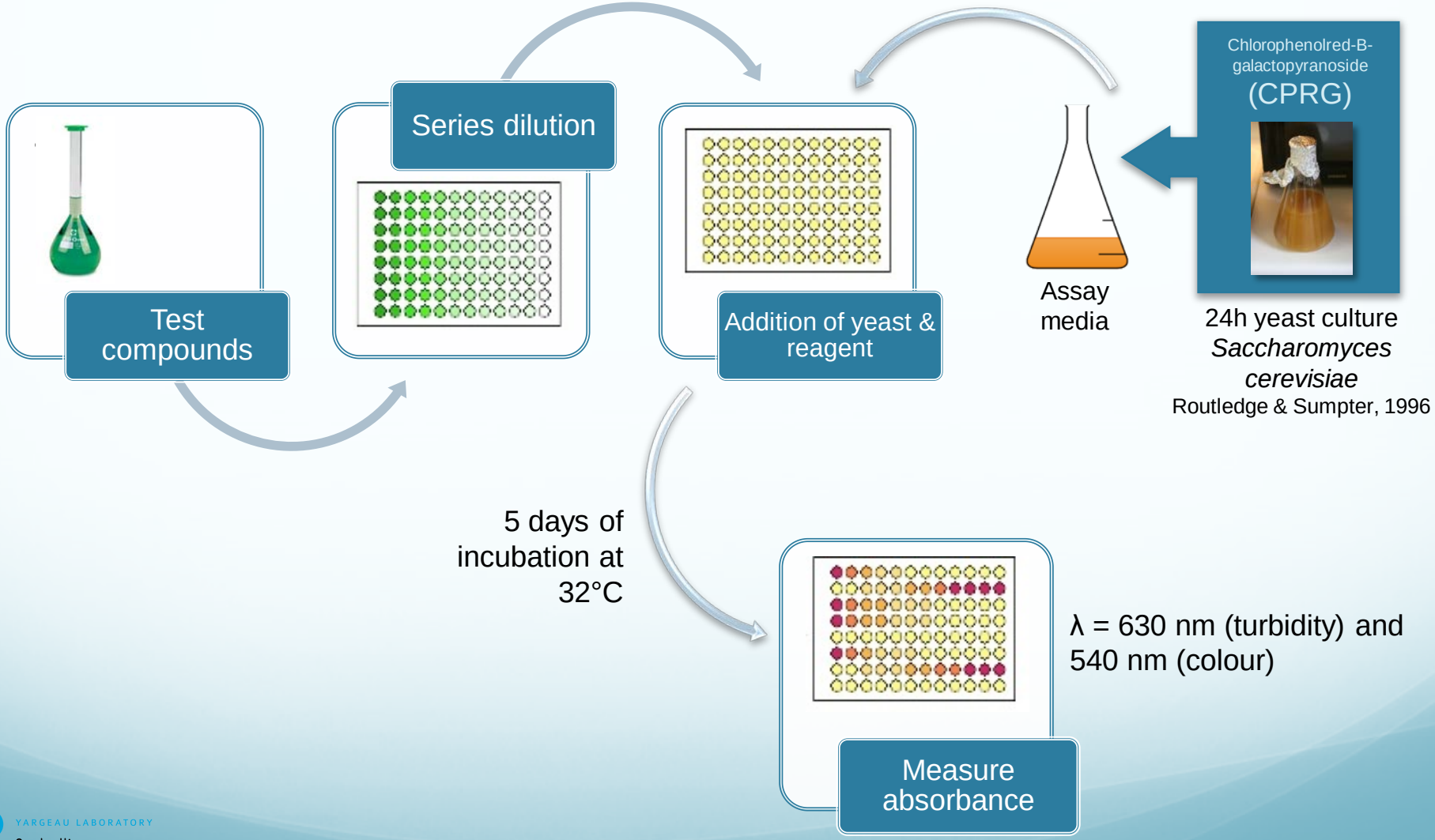




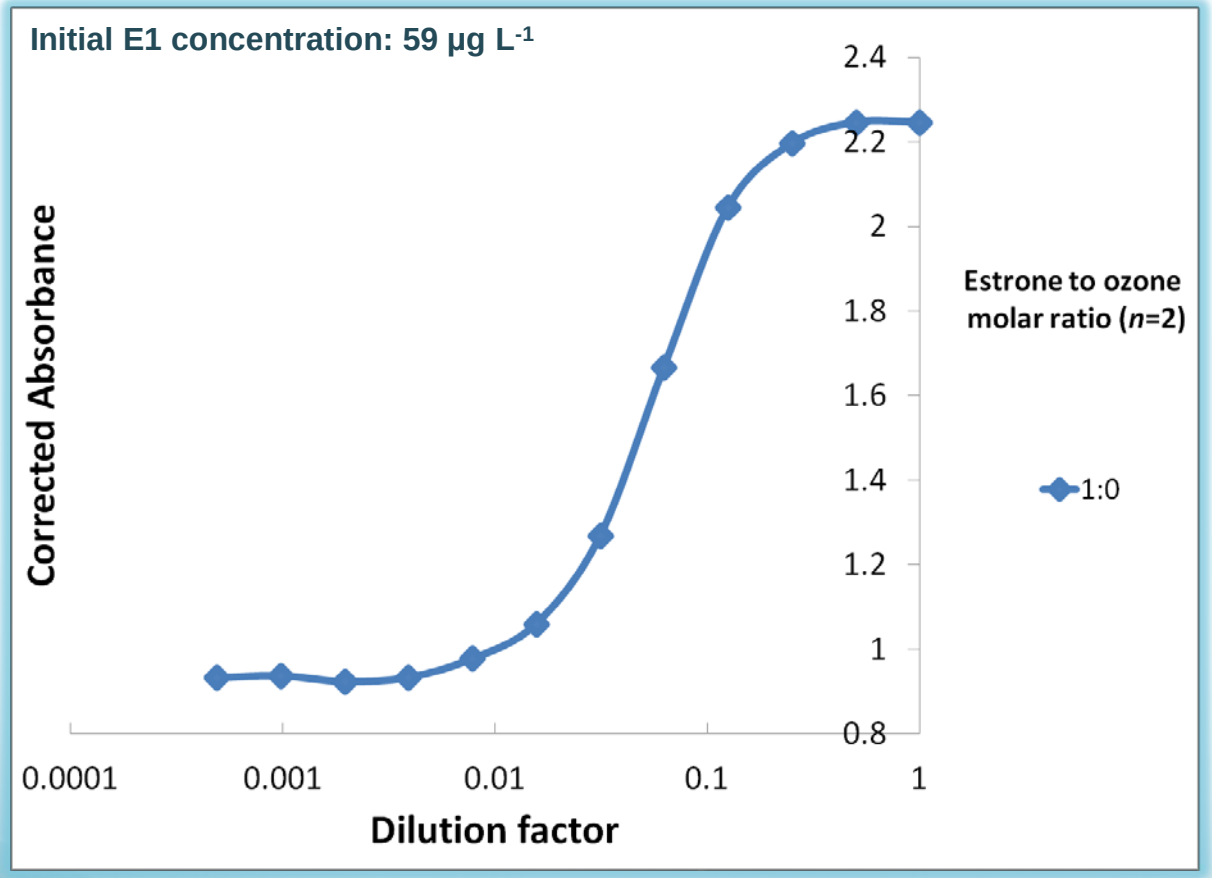
Accela LC system coupled to a LTQ Orbitrap XL
Thermo Fisher Scientific

Sieve 1.3
Xcalibur 2.1
Mass Frontier 7.0

Yeast estrogenic screen (YES) assay



Estrogenic activity of ozonated E1 samples



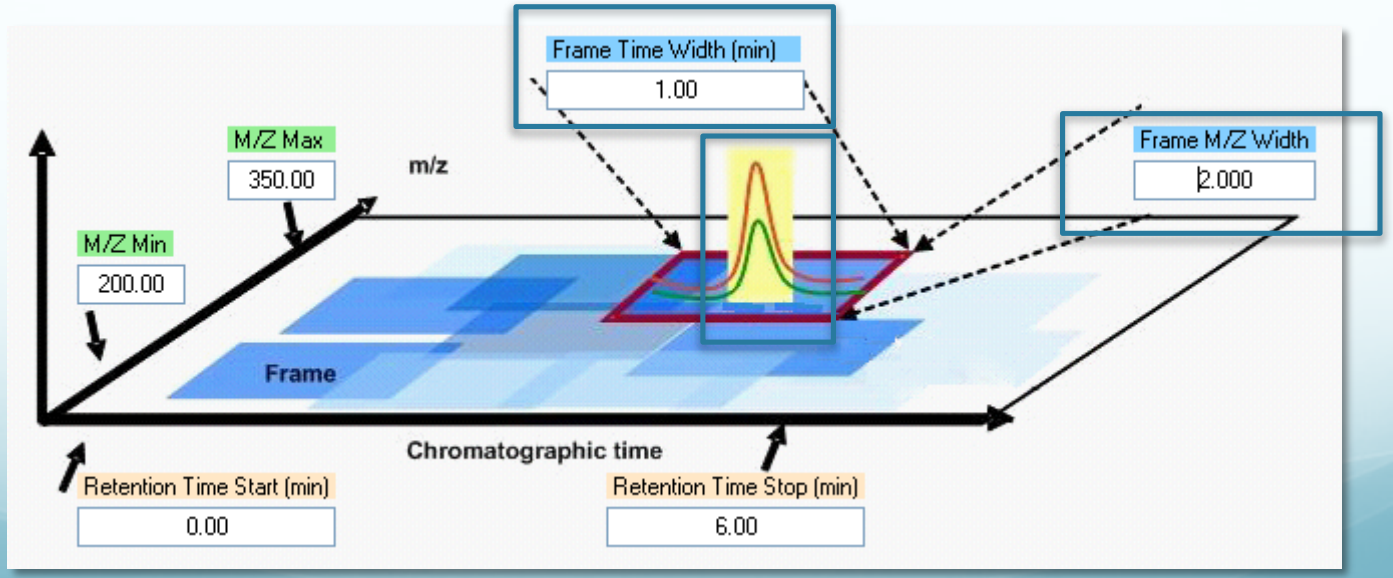
SAMPLE $E1:O_3$	E1 REMOVAL
1:0	0%
2:1	1%
1:1	11%
1:2	55%
1:4	75%
1:8	91%

Estrogenic activity

Slight initial increase at lower molar ratio doses (2:1 and 1:1) followed by a decrease at higher molar ratios

Identification of TPs

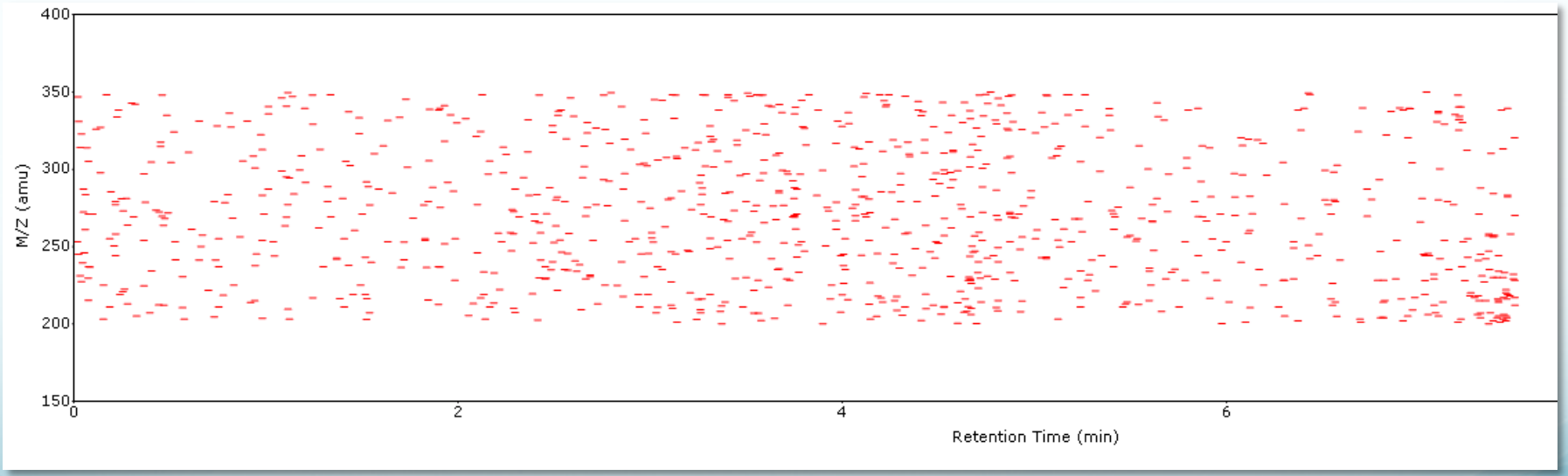
- **Use of SIEVE (Thermo Scientific) along with a "Control Compare Trend" experiment to eliminate background signals from the sample and identify potential unknown ozonation**
- **The software allows extraction of signals from the acquisition files using a three coordinate approach: *retention time*, *m/z value* and *intensity*. This 3D space is called a “frame”**



Example of a SIEVE frame

Identification of TPs – SIEVE results

- **Samples having different E1 to O_3 ratios were analyzed with LTQ Orbitrap XL in both APCI(+) and APCI(-)**
  - Acquisition files were processed with SIEVE.
  - SIEVE found 593 frames for the trend analysis of the APCI- files.

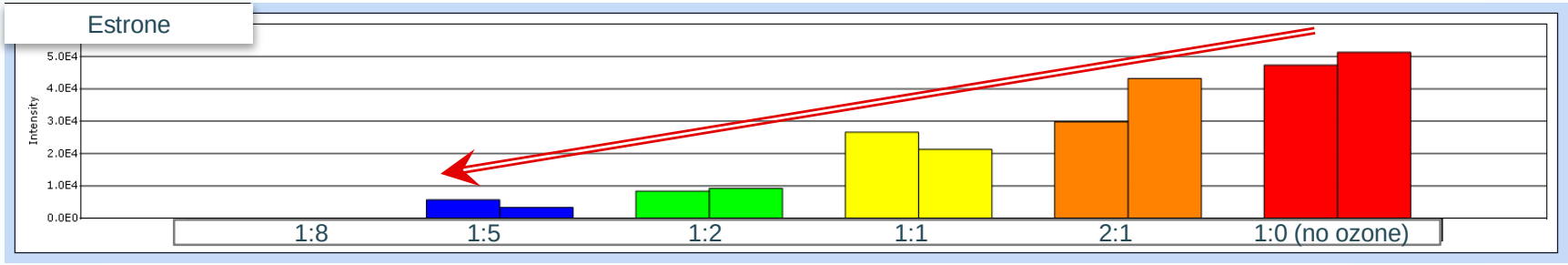


"Gel view" in SIEVE of the frames found in the E1:O3 samples (APCI+)

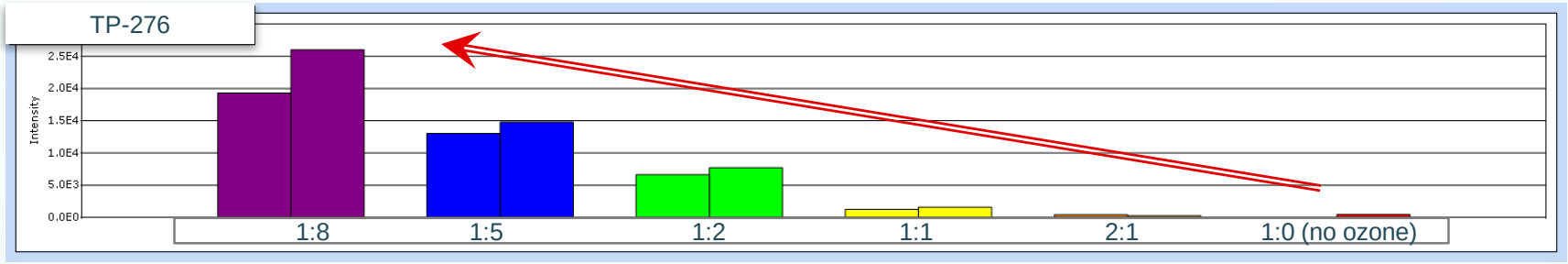
Applying rules to select frames

- **Ratio of intensities of a frame**
  - sample 1:0 to sample 1:8 (control) must be < 0.05
    - This rule ensure that frames has very low intensity in non-ozonated sample
- **Ratio of 1:1/1:8 and ratio of 1:5/1:8 must be > 0.05**
    - This second rule guarantee that the filtered frames are not associated to random signal spikes since they have to be present in two samples exposed to different O_3 doses
- **The average intensity of the frame in the sample 1:8 must be > 5000**
  - To target more abundant TPs and with sufficient intensity for $HRMS^n$

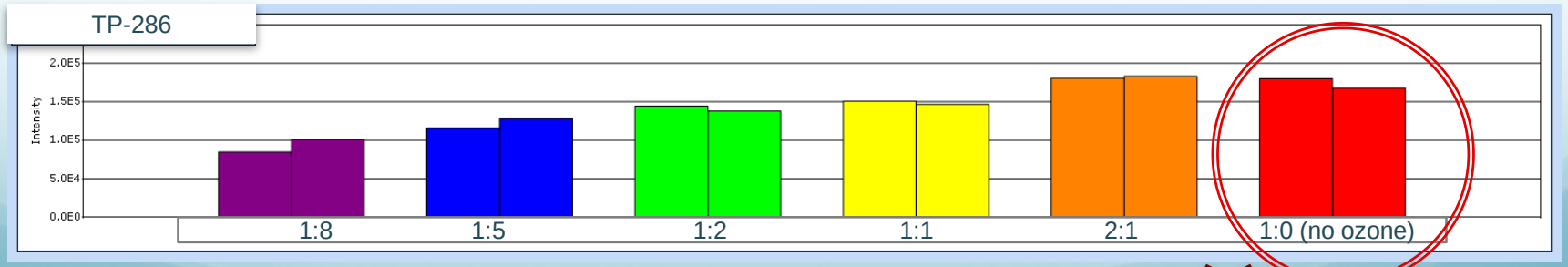
Applying rules to select frames



Trend analysis of frame #4 (269.15533 m/z) which corresponds to estrone



Trend analysis of frame #9 (275.12930 m/z) which corresponds to TP-276



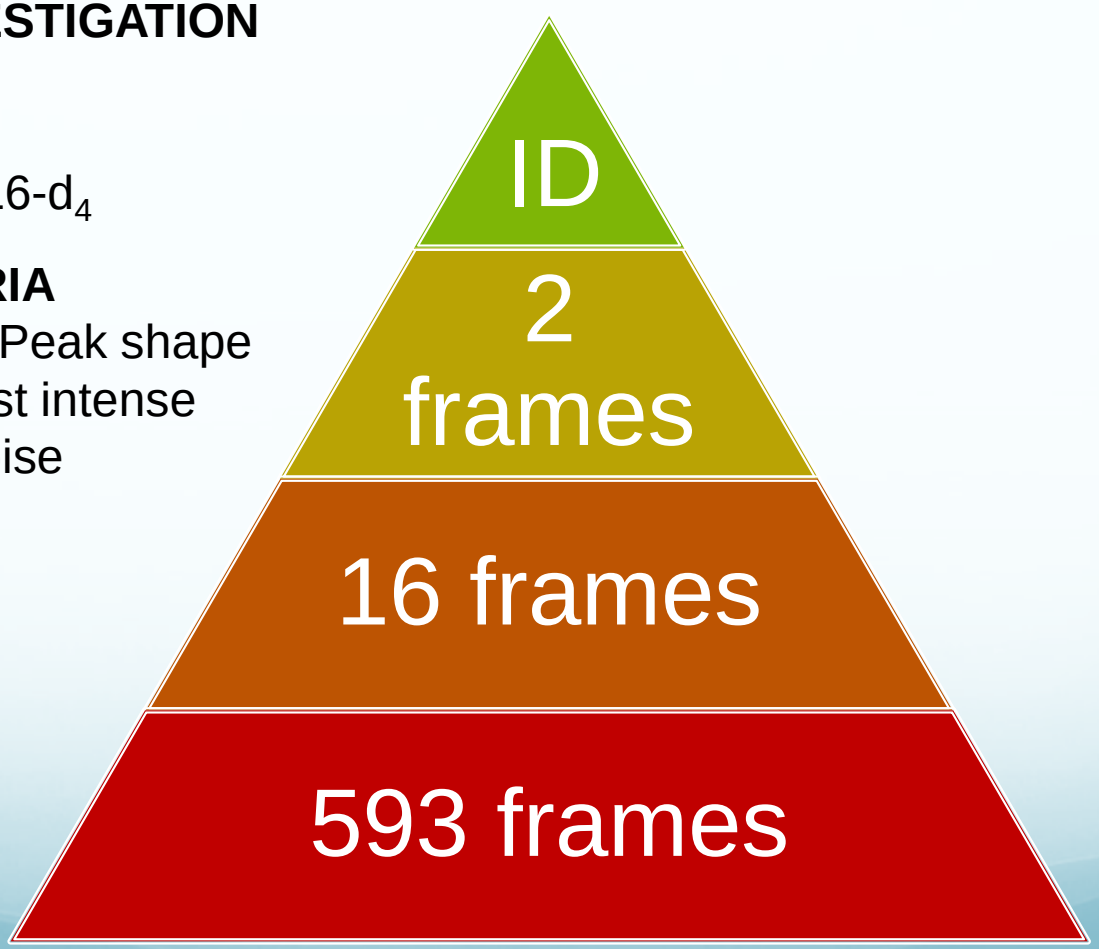
Trend analysis of frame #1 (285.15033 m/z) which corresponds to TP-286

Filtered frame results

FURTHER INVESTIGATION
Xcalibur
$HRMS^n$
Estrone-2,4,16,16-d_4

OTHER CRITERIA
RT consistency, Peak shape
Part of 100^{th} most intense
Low signal-to-noise

SIEVE
Rules & Trend analysis



Elemental composition of TPs

- **Using QualBrowser program of Xcalibur 2.1**

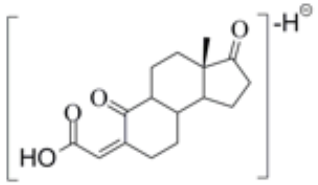
Frame #	*m/z*	Elemental composition	RDBE	Δmmu
E1	269.15537	$C_{18}H_{21}O_2$	8.5	0.667
#9	275.12930	$C_{16}H_{19}O_4$	7.5	0.418
#78	317.14008	$C_{18}H_{21}O_5$	8.5	0.633
		$C_{11}H_{25}O_{10}$	-0.5	-5.240

RDBE: Ring and double bond equivalents
Δmmu: experimental error in milimass units

loss of 2 C and 2 H atoms and addition of 2 O

Most likely structure: addition of 3 O

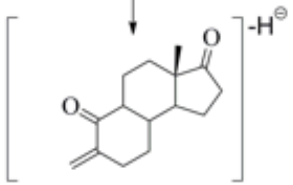
OTP-276



$C_{16}H_{19}O_4^-$
$m/z_{(exp)}$= 275.12906
Δmmu=0.178

MS^1

-(CO_2)



$C_{15}H_{19}O_2^-$
$m/z_{(exp)}$= 231.13989
Δmmu=0.837

MS^2

-($\dot{H}$ + CH_4)

-(H_2 + CH_4)

-(2 CH_4)

-(C_6H_8O)

-($C_6H_{10}O$)

$C_9H_{11}O^-$
$m/z_{(exp)}$= 135.08226
Δmmu=0.722

$C_{14}H_{13}O_2^-$
$m/z_{(exp)}$= 213.09290
Δmmu=0.797

$C_{14}H_{14}O_2^{\bullet-}$
$m/z_{(exp)}$= 214.10075
Δmmu=0.822

$C_{13}H_{11}O_2^-$
$m/z_{(exp)}$= 199.07718
Δmmu=0.727

$C_9H_9O^-$
$m/z_{(exp)}$= 133.06660
Δmmu=0.712

MS^3

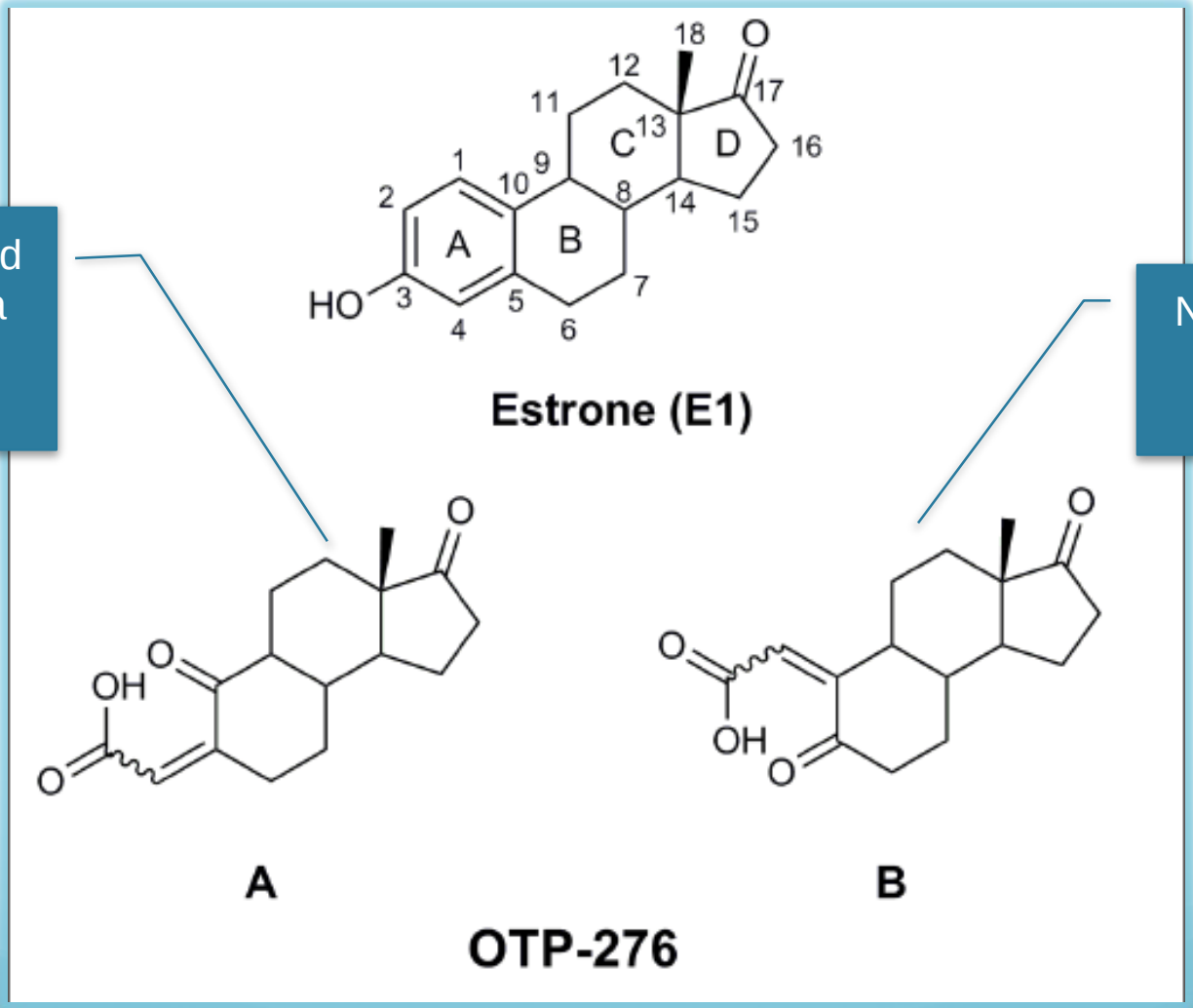
-($\dot{C}H_3$)

$C_{13}H_{11}O_2^-$
$m/z_{(exp)}$= 199.07718
Δmmu=0.964

MS^4

Structural elucidation by HRMS^n

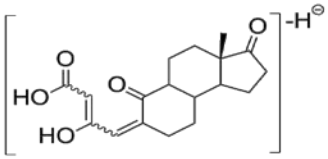
Also suggested by de Oliveira Pereira et al. (2011)



Not observed in ozonated E1-d_4

Estrone-2,4,16,16-d_4

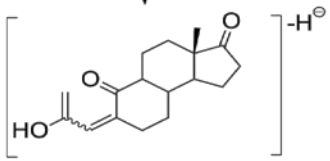
OTP-318



$C_{18}H_{21}O_5^-$
$m/z_{(exp)}$= 317.13981
Δmmu=0.363

MS^1

$-(CO_2)$



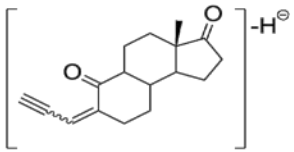
$C_{17}H_{21}O_3^-$
$m/z_{(exp)}$= 273.14960
Δmmu=-0.018

MS^2

$-(C_6H_8O)$ | $-(CO)$ | $-(H_2O)$ | $-(C_2H_2O_2)$ | $-(C_8H_8O_2)$

$C_{11}H_{13}O_2^-$
$m/z_{(exp)}$= 177.09214
Δmmu=0.037

$C_{16}H_{21}O_2^-$
$m/z_{(exp)}$= 245.15460
Δmmu=-0.103



$C_{17}H_{19}O_2^-$
$m/z_{(exp)}$= 255.13896
Δmmu=-0.298

$C_{15}H_{19}O^-$
$m/z_{(exp)}$= 215.14396
Δmmu=-0.179

$C_9H_{13}O^-$
$m/z_{(exp)}$= 137.09735
Δmmu=0.162

MS^3

$-(C_3H_4O)$ | $-(H_2O)$ | $-(\dot{C}H_3)$ | $-(C_8H_8O)$

$C_{14}H_{15}O^-$
$m/z_{(exp)}$= 199.11260
Δmmu=-0.288

$C_{17}H_{17}O^-$
$m/z_{(exp)}$= 237.12804
Δmmu=-0.499

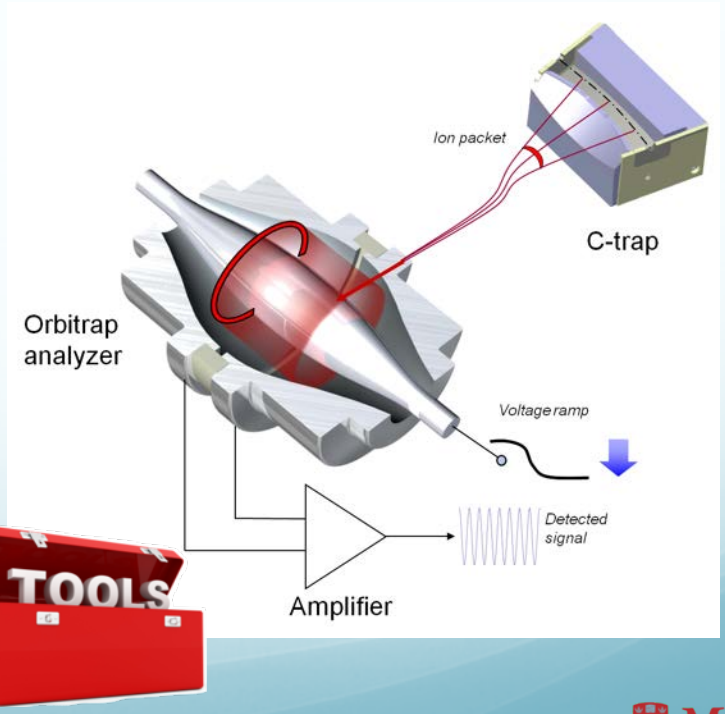
$C_{16}H_{16}O_2^-$
$m/z_{(exp)}$= 240.11510
Δmmu=-0.478

$C_{11}H_{11}O^-$
$m/z_{(exp)}$= 159.08142
Δmmu=-0.118

MS^4

Is a mass spectrometer part of an engineer's toolbox?

- **The approach**
  - allows an efficient identification of TPs
  - further supports that monitoring the removal of parent compounds is not sufficient
  - suggests that the main TPs formed could not explain the initial increase in estrogenic activity (unless important TPs were eliminated)



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