Simultaneous Quantitative and Qualitative Identification of Transformation Products of Triamcinolone Acetonide by Ozonation Using Liquid Chromatography (LC) Hyphenated with Quadrupole Time-of-Flight Mass Spectrometry (Q-TOF-MS)

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TOrCs in wastewater

Ubiquity of TOrCs in wastewater

- Trace organic contaminants (TOrCs) occur in wastewater ubiquitously.

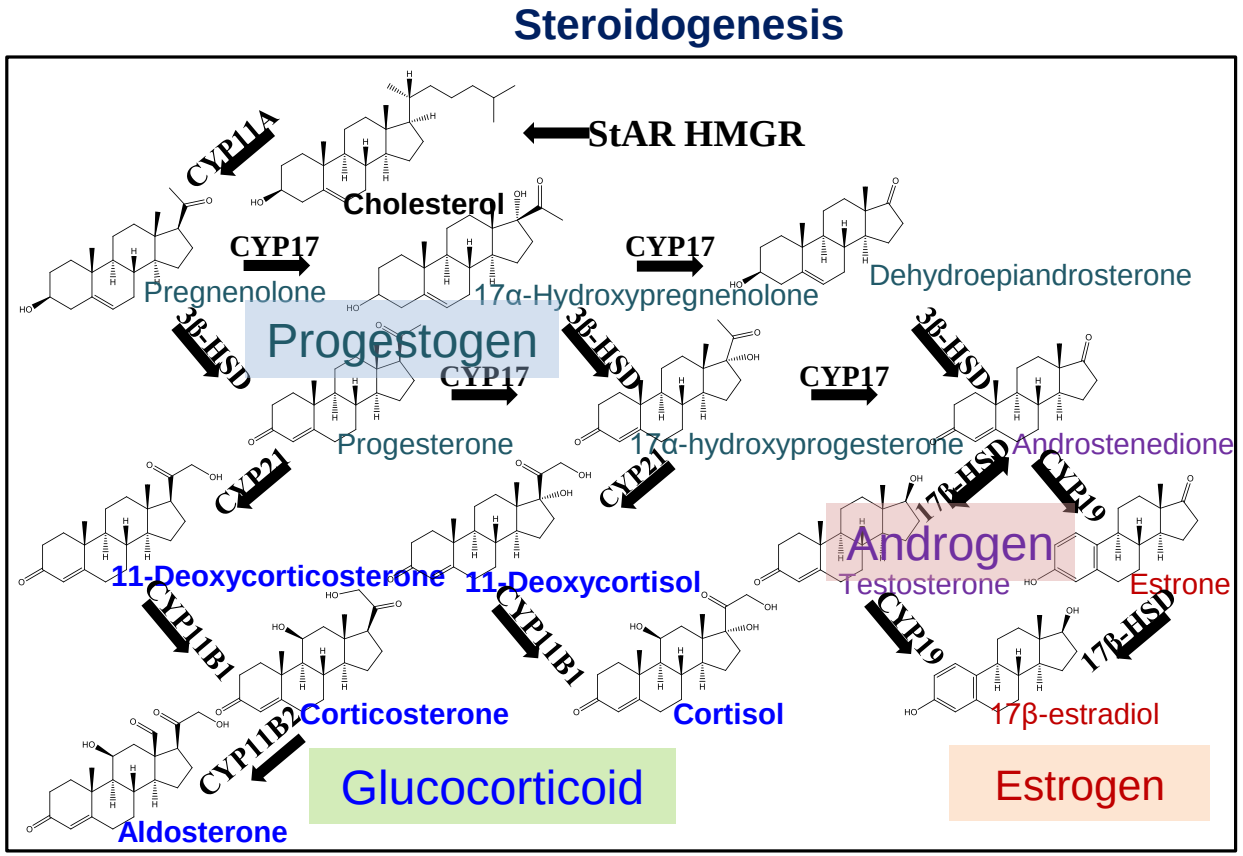
- Conventional WWTP is not efficacious for the attenuation of TOrCs.

- *Pharmaceuticals*
- *Personal care products*
- *Steroid hormones*
- *Industrial chemicals*

Glucocorticoids (GCs) are one class of steroid hormones in vertebrates

- Glucocorticoids (GCs) affect energy metabolism, immune system response, and stress adaption in vertebrates.

- GCs show effects mainly by binding to the nuclear glucocorticoid receptor (GR) and subsequent regulation of related gene expression.



GCs are top prescribed medicines

Amount prescribed in UK (2006)

Class	Prescribed (kg)	
Estrogens	488.79	9 times
Androgens	306.62	14 times
Progestogens	1704.65	3 times
Glucocorticoids	4367.72	

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Pharmaceuticals in the Aquatic Environment: Steroids and Anti-Steroids as High Priorities for Research

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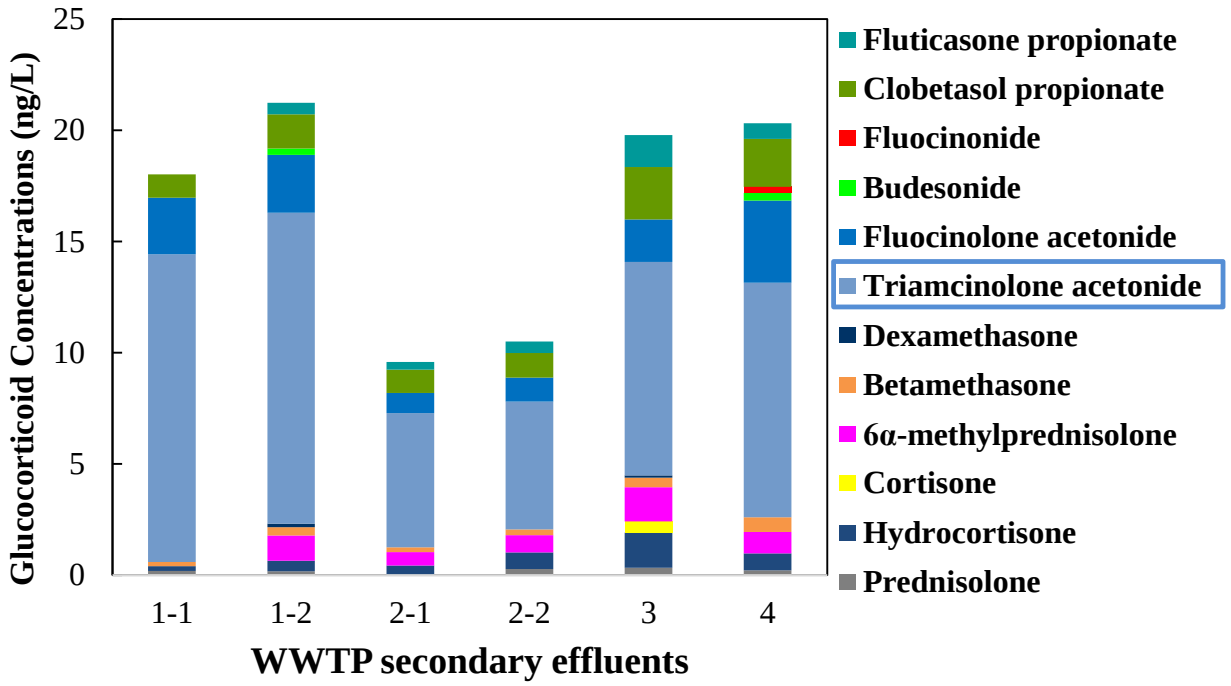
Published online: 15 Dec 2010.

Five medicines with GCs were listed in top 100 most prescribed drugs in USA (2013)

Name	Brand	by sales	by units
Fluticasone Propionate	Advair Diskus	6	6
	Flovent HFA	52	32
Budesonide	Uceris	57	1
	Symbicort	30	26
Mometasone	Nasonex	46	24

Source: drug.com (database: IMS)

Glucocorticoids in WWTP effluents



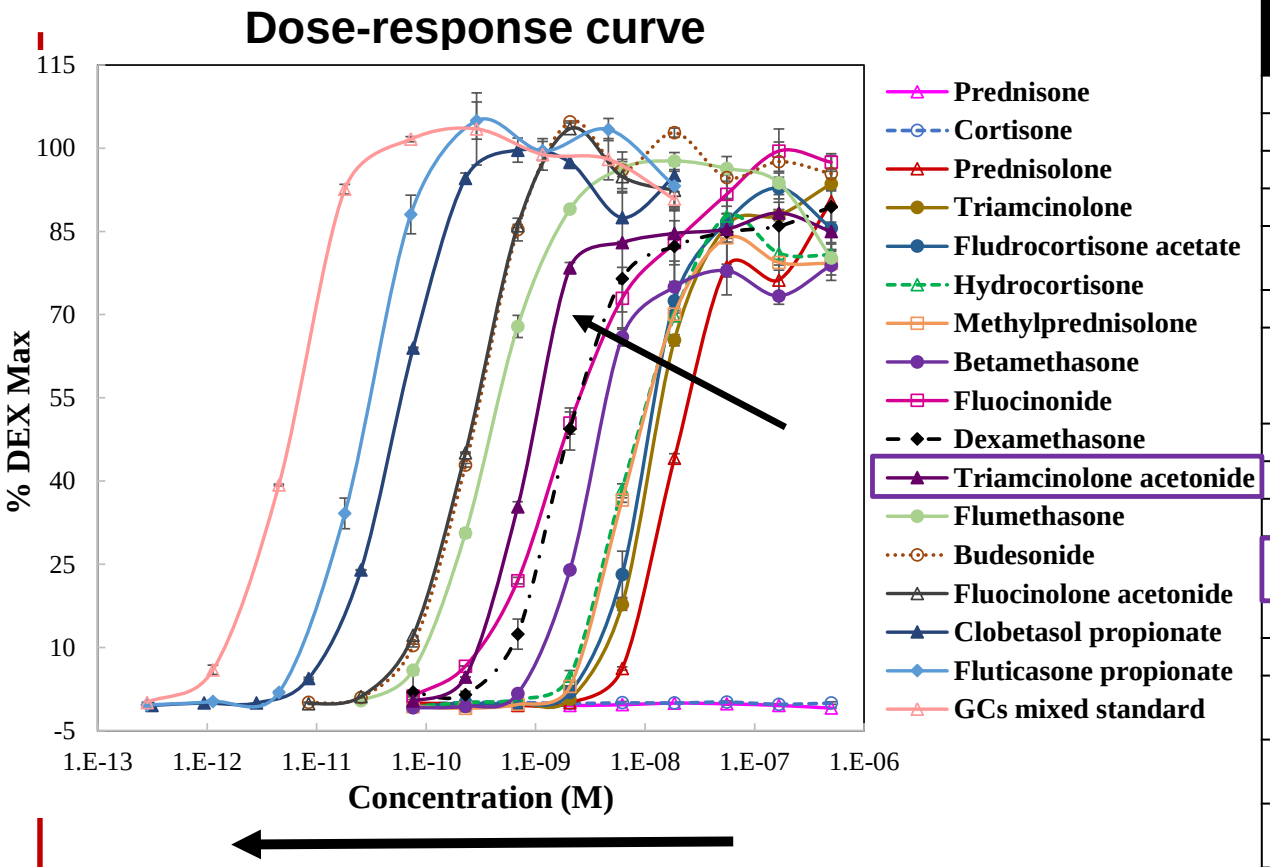
49-77% of the total GCs

5.75 -14.0 ng/L

Higher occurrence level than estrogens (<1 ng/L)

Environ. Sci. Technol., 2016, 50 (6), 2870–2880.

Relative potency of glucocorticoids



GCs	EC_{50} (nM)	REP
Prednisone	>500	<0.004
Cortisone	>500	<0.004
Prednisolone	17.7	0.101
Triamcinolone	11.8	0.152
Fludrocortisone acetate	9.67	0.185
Hydrocortisone	6.81	0.264
6α-methylprednisolone	6.79	0.264
Betamethasone	2.83	0.634
Fluocinonide	1.89	0.948
Dexamethasone	1.79	1.000
Triamcinolone acetonide	0.79	**2.265**
Flumethasone	0.36	5.032
Budesonide	0.26	6.895
Fluocinolone acetonide	0.24	7.398
Clobetasol propionate	0.048	37.04
Fluticasone propionate	0.025	70.88
GCs mixed standard	0.005	329

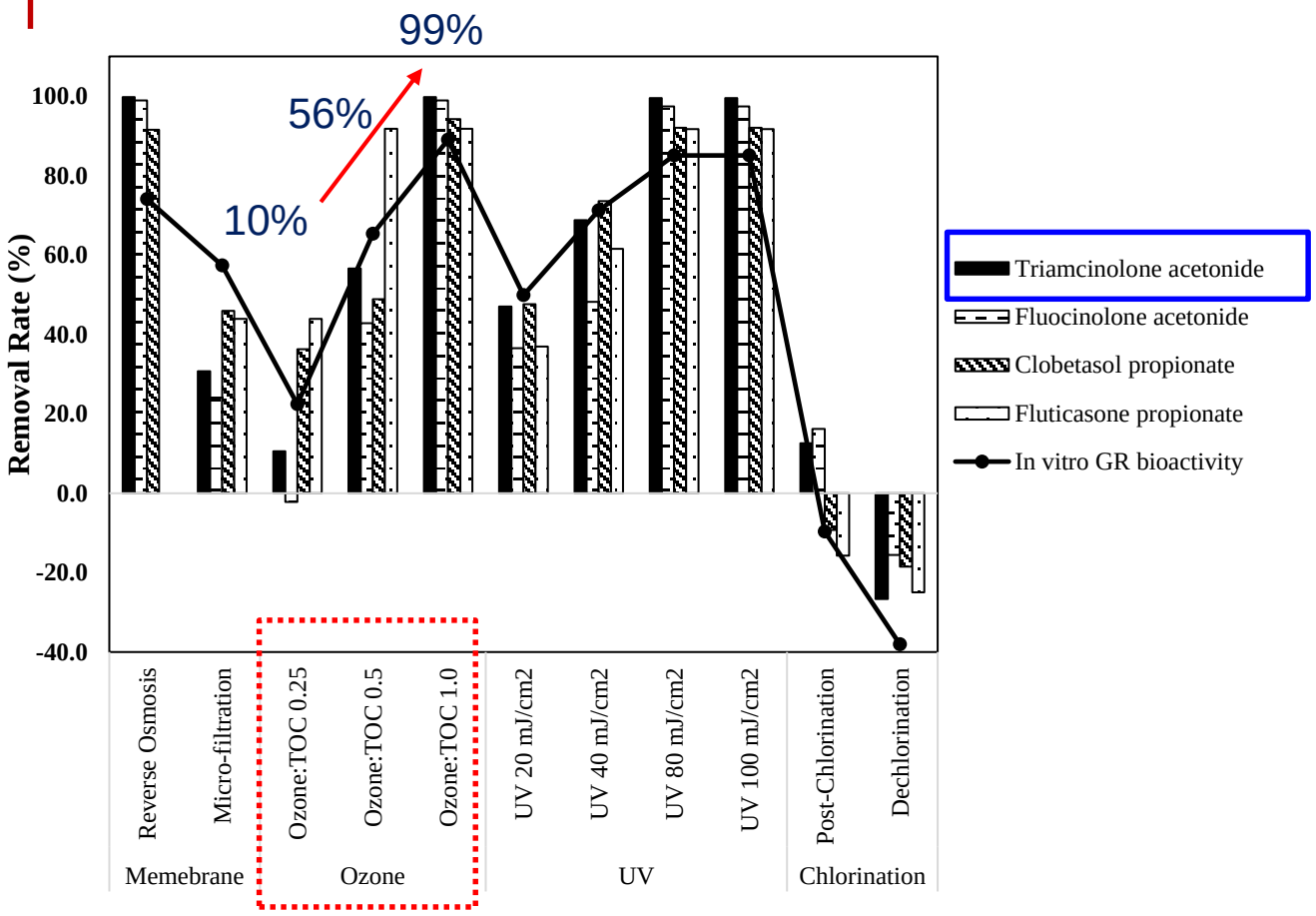
Most synthetic GCs have much higher activity than natural GCs

Environ. Sci. Technol., 2016, 50 (6), 2870–2880.

An increasing release of certain GCs is expected

- Triamcinolone acetonide (TA) and fluticasone propionate (FP) are approved as **over-the-counter (OTC) drugs** by US FDA since 2014.

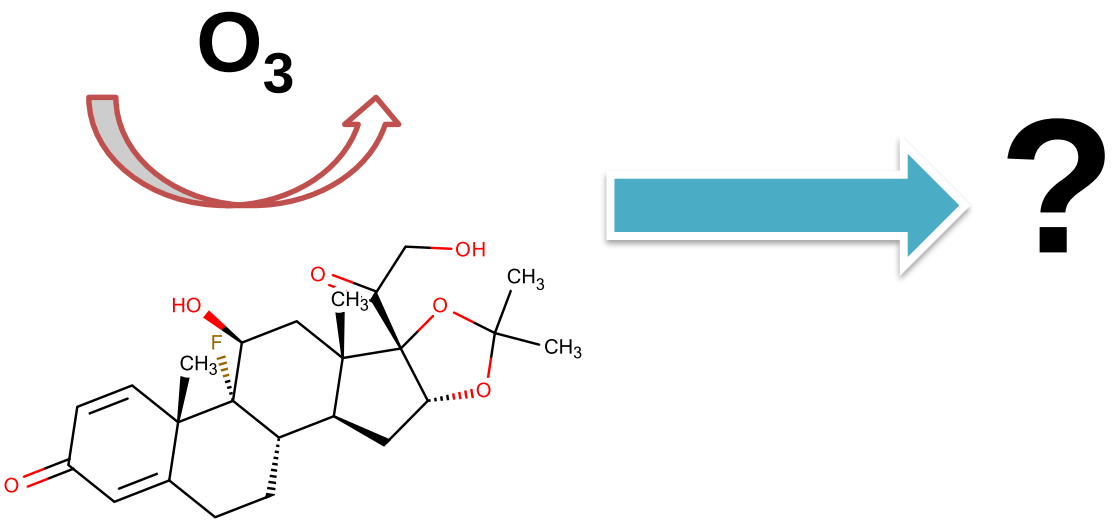
Attenuation of several GCs via advanced water treatment processes



- RO and UV were efficacious for GC attenuation
- Chlorination cannot remove GCs
- The attenuation of GCs by ozonation is dependent on the applied ozone dose

Environ. Sci. Technol., 2016, 50 (6), 2870–2880.

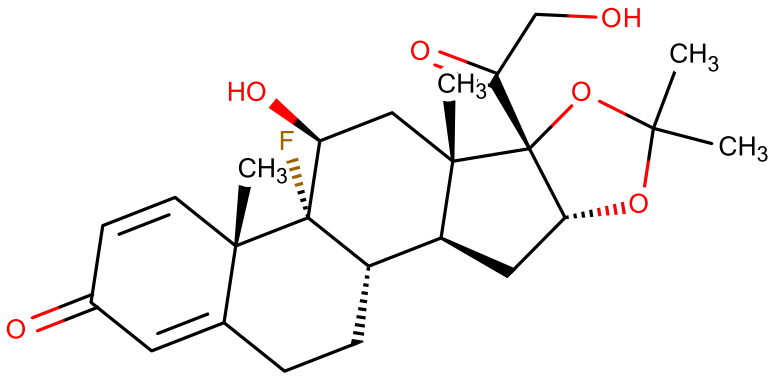
Transformation products



Objectives

- To identify transformation products of GCs by ozone oxidation quantitatively and qualitatively.
- To provide comprehensive data analysis procedure for identification of unknown transformation compounds

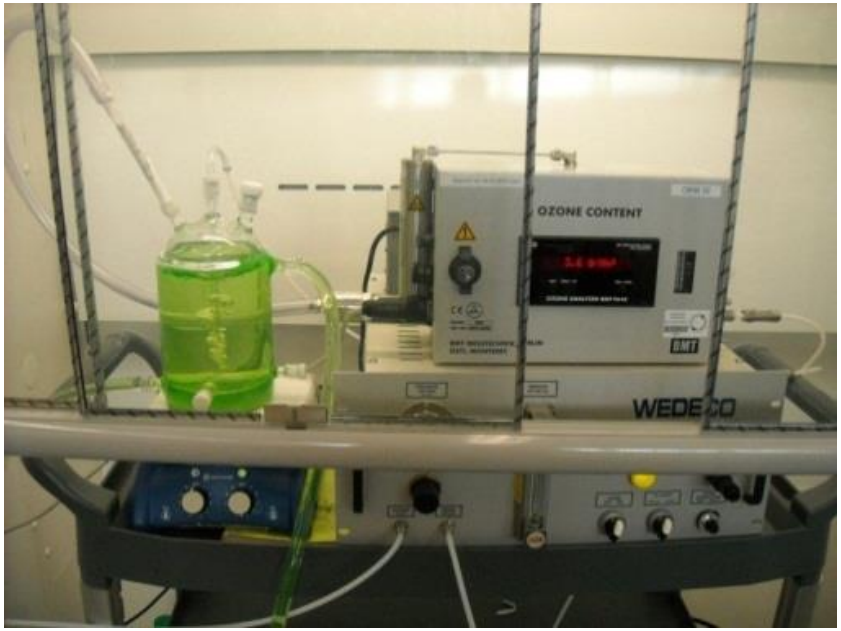
Triamcinolone acetonide (TA)



Formula: $C_{24}H_{31}FO_6$
Monoisotopic weight: 434.2104
Log K_{ow}: 2.53

Ozonation experiment

Generation of ozone stock solution

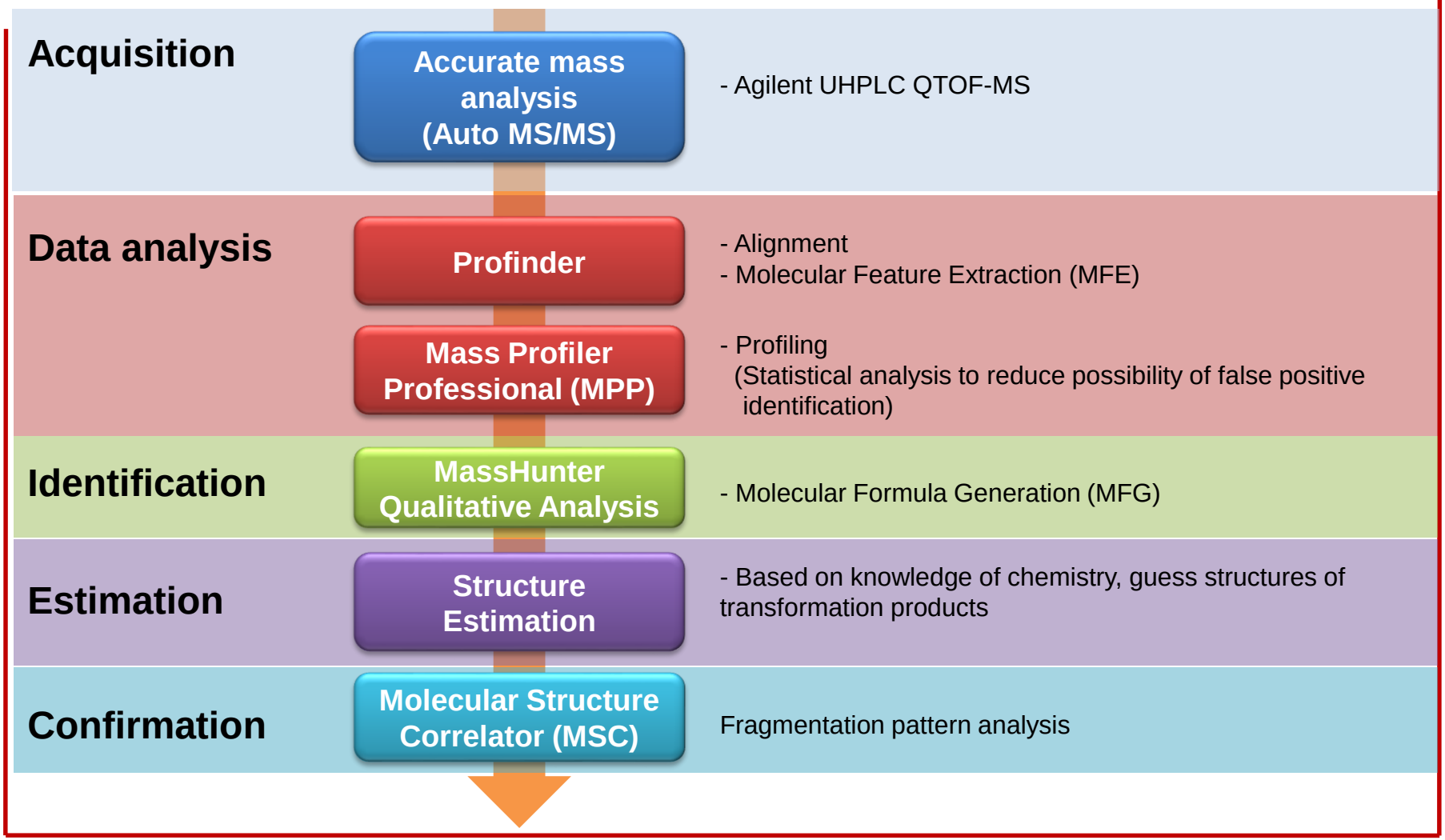


< Xylem Wedeco 8HC modular >

Ozone concentrations:
0, 0.8, 2, 4 & 8 mg/L

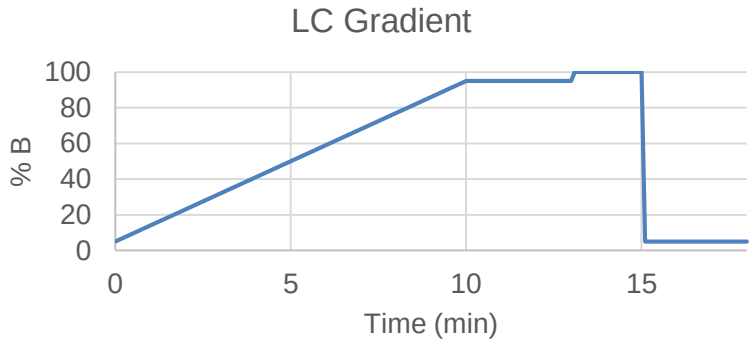
4 mM t-BuOH

Work flow


Acquisition
Accurate mass analysis (Auto MS/MS)
- Agilent UHPLC QTOF-MS
Data analysis
Profinder
- Alignment
- Molecular Feature Extraction (MFE)
Mass Profiler Professional (MPP)
- Profiling
(Statistical analysis to reduce possibility of false positive identification)
Identification
MassHunter Qualitative Analysis
- Molecular Formula Generation (MFG)
Estimation
Structure Estimation
- Based on knowledge of chemistry, guess structures of transformation products
Confirmation
Molecular Structure Correlator (MSC)
Fragmentation pattern analysis

Instrumentation

LC run parameters	
Column	Agilent Eclipse C18, 2.1 × 50 mm, 1.8 µm
Column Temp.	30 ^{o}C
Injection Volume	40 µL
Mobile Phase	H_2O-0.1% acetic acid (A) Acetonitrile (B)



Agilent UHPLC-QTOF-MS (6540)

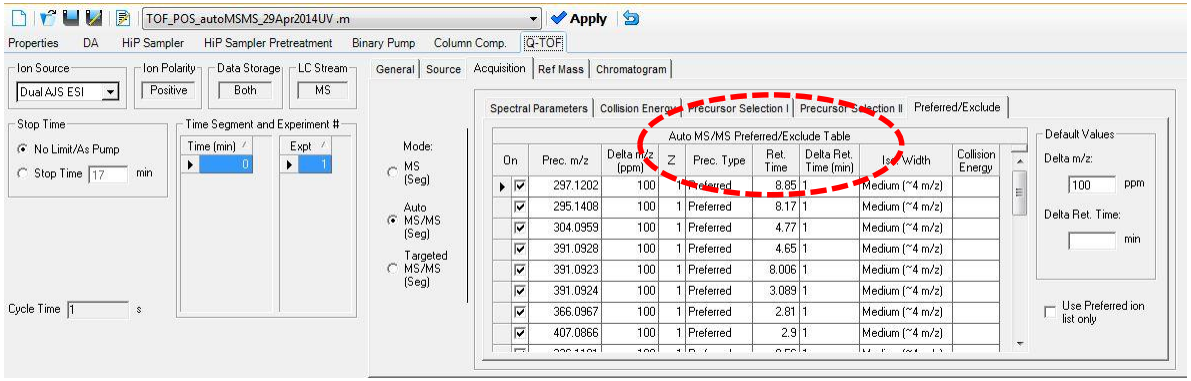
Instrumentation

MS run parameters	
Source	Dual AJS ESI-negative
Reference masses	112.9896, 1033.9881
MS/MS scan mode	Auto MS/MS
Collision energy (V)	10, 20, 40
Fragmentor (V)	110
Gas temperature (°C)	250
Sheath Gas Temperature (°C)	300
Sheath Gas Flow (L/min)	11
MS Min Range (m/z)	50
MS Max Range (m/z)	1100
MS Scan Rate (spectra/sec)	2
MS/MS Scan Rate (spectra/sec)	5

Auto MS/MS

- Automatic MS/MS triggers MS/MS mode from MS scan mode if ions are detected above a threshold abundance (20000 counts/spectrum used in this study)

- Advantage
  - Efficient because MS scan and MS/MS can be done in one run.



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Profiling

Mass Profiler Professional

234

- Number of features identified after **alignment of data**.

215

- Number of features after **frequency filtering** with 2/3 of each group of samples

102

- **ANOVA** at 95% confidence level, **Bonferroni test** and **Tukey test**
- Number of features that are statistically significant

54

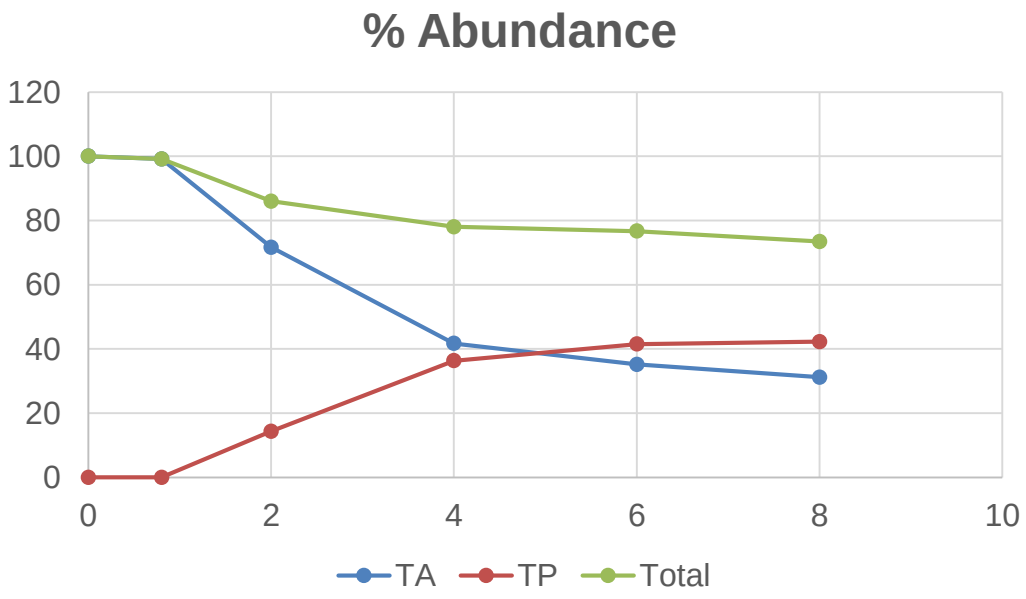
- **Fold change (4 fold)**
- Number of features that have ion counts that changed by at least a factor of 4 between $PreO_3$ and $PostO_3$

9

- Number of features that have **MFG score > 80**

Profiling results

- ~75% of transformation products by abundance can be explained by the statistical profiling method.



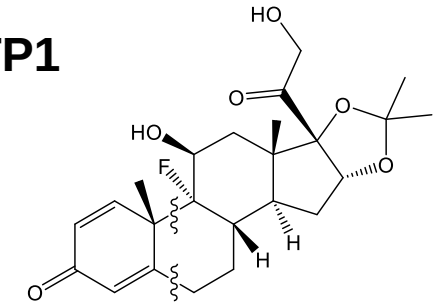
Profiling results

List of TPs

	Formula	Score	Mass	Diff (ppm)	RT
TP1	**$C_{23}H_{31}FO_8$**	**95.7**	**454.2002**	**0.23**	**5.176**
TP2	$C_{23}H_{29}FO_8$	92.54	452.1856	-2.1	4.709
TP3	$C_{19}H_{34}O_{12}$	91.69	454.205	0.05	5.176
TP4	$C_{27}H_{30}O_7$	97.16	466.1984	1.58	3.98
TP5	$C_{20}H_{34}FO_{14}$	87.54	517.1957	-4.74	4.374
TP6	$C_{27}H_{42}O_{15}$	93.34	606.2506	2.99	3.98
TP7	$C_{21}H_{36}FO_{15}$	83.93	547.2063	-4.56	3.975
TP8	$C_{30}H_{29}FO$	81.9	424.2194	1.96	4.696
TP9	$C_{29}H_{36}O_{10}$	85.19	544.2296	2.27	4.782

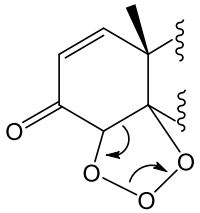
Proposed transformation pathway

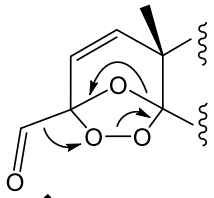
TP1



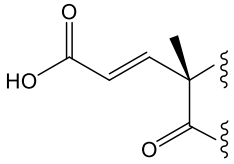
$C_{24}H_{31}FO_6$

+O_3

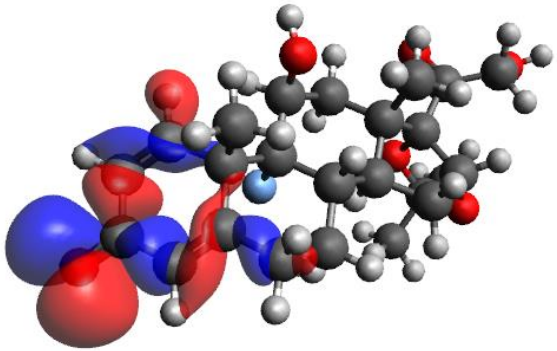




-CO



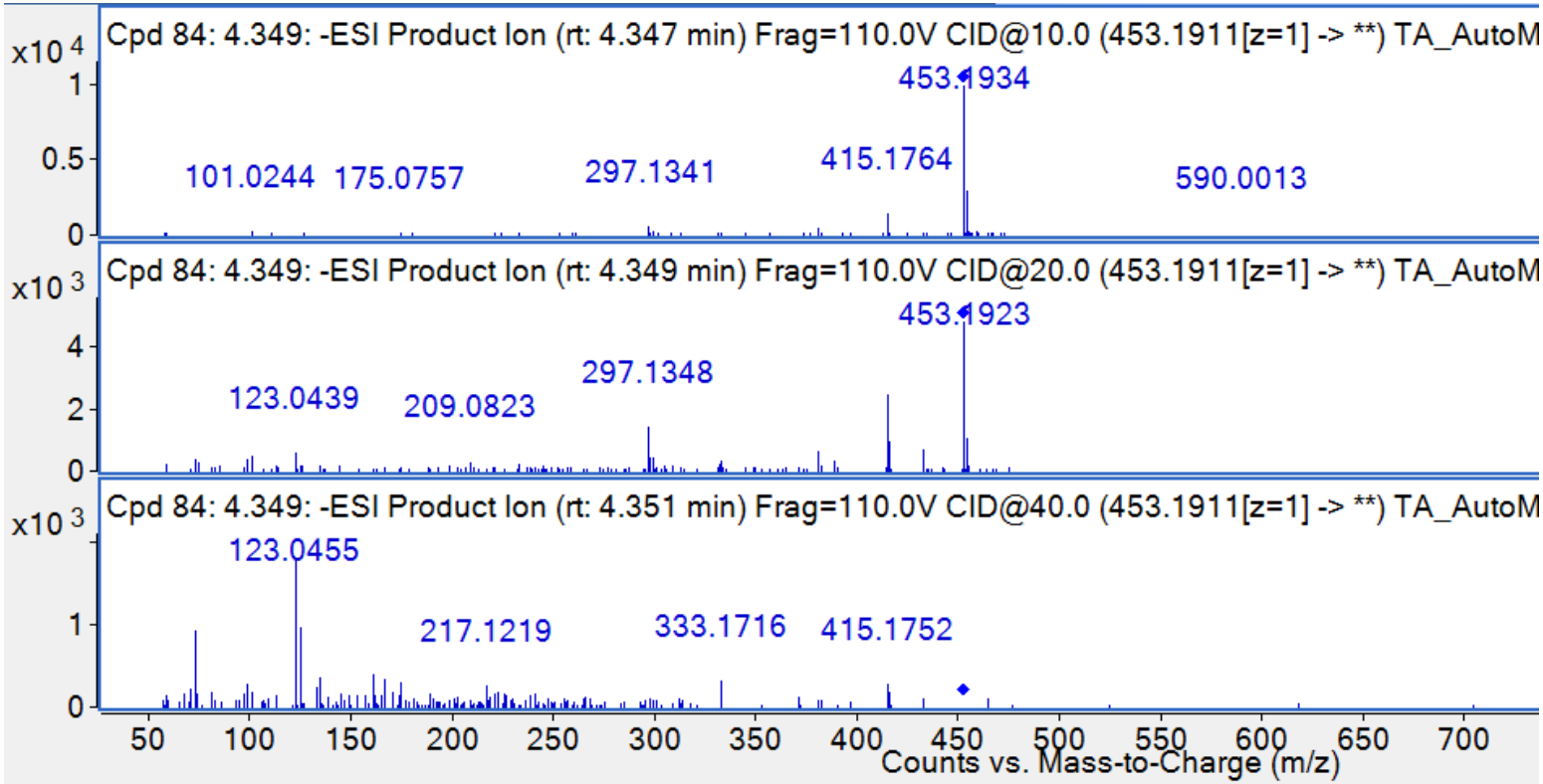
$C_{23}H_{31}FO_8$



HOMO

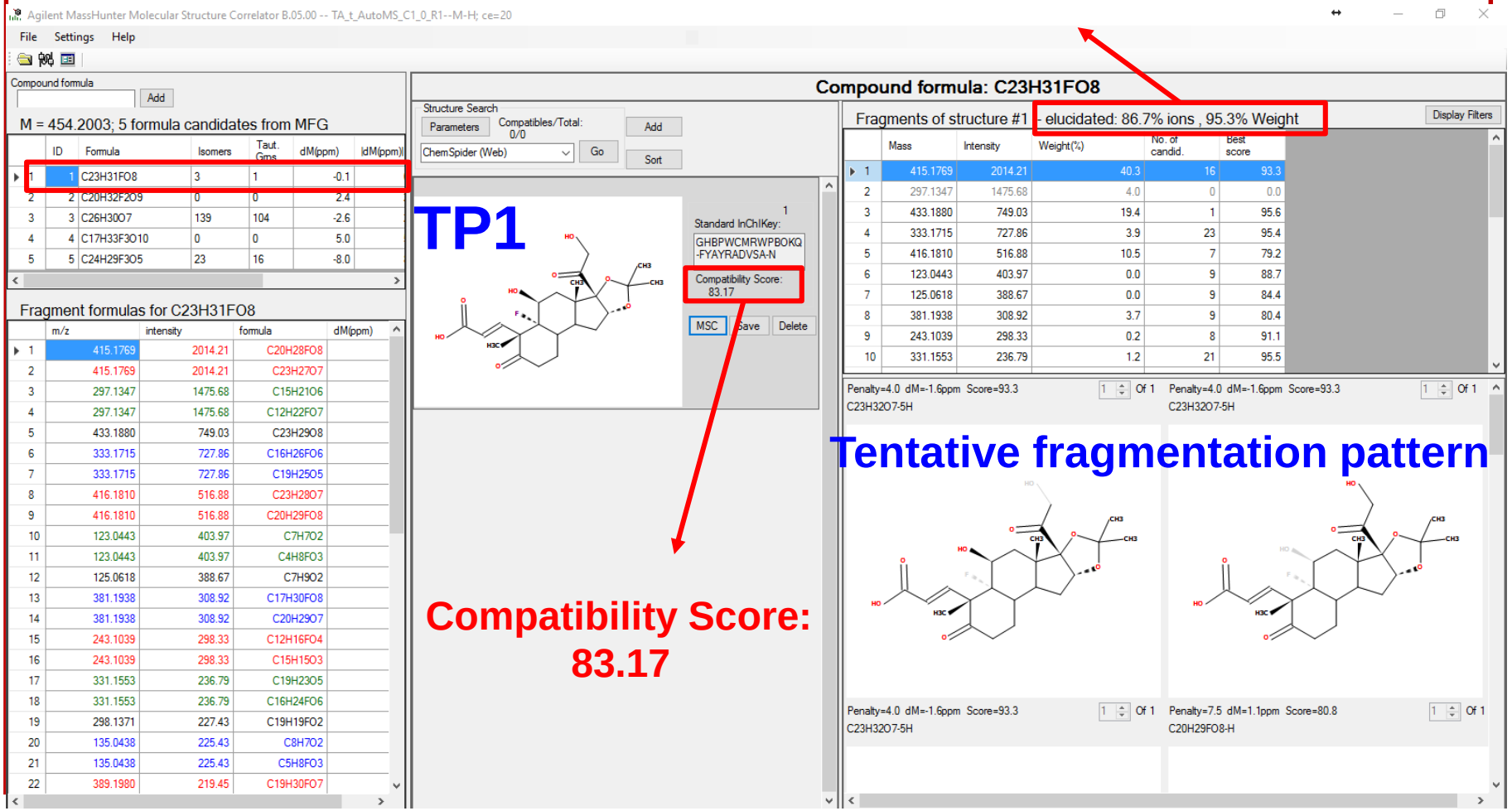
Fragmentation pattern of TP1

$C_{23}H_{31}FO_8$



MSC

Elucidated: 86.7% ions, 95.3% weights



Conclusion

- Transformation products (TPs) of triamcinolone acetonide (TA) by ozone oxidation was identified quantitatively and qualitatively using Auto MS/MS.
- With a single run, both quantitative (abundance) and qualitative (formula and fragmentation pattern) information could be obtained via Auto MS/MS.
- A statistical profiling tool efficiently screened insignificant TPs.
- Molecular structure correlator (MSC) can be a useful tool to identify fragmentation pattern.

Acknowledgement

- Snyder Research group (University of Arizona)
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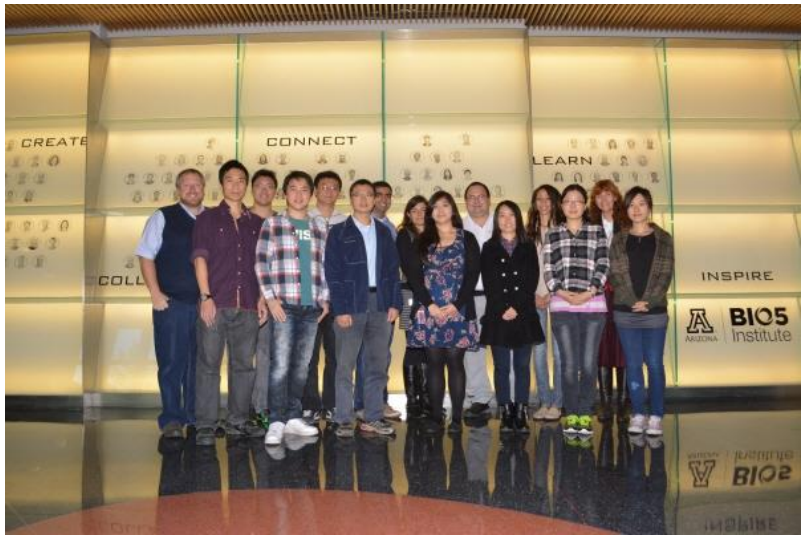
- The Metropolitan Water District of Southern California
  - Dr. Ai Jia



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Q&A

Questions???