



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

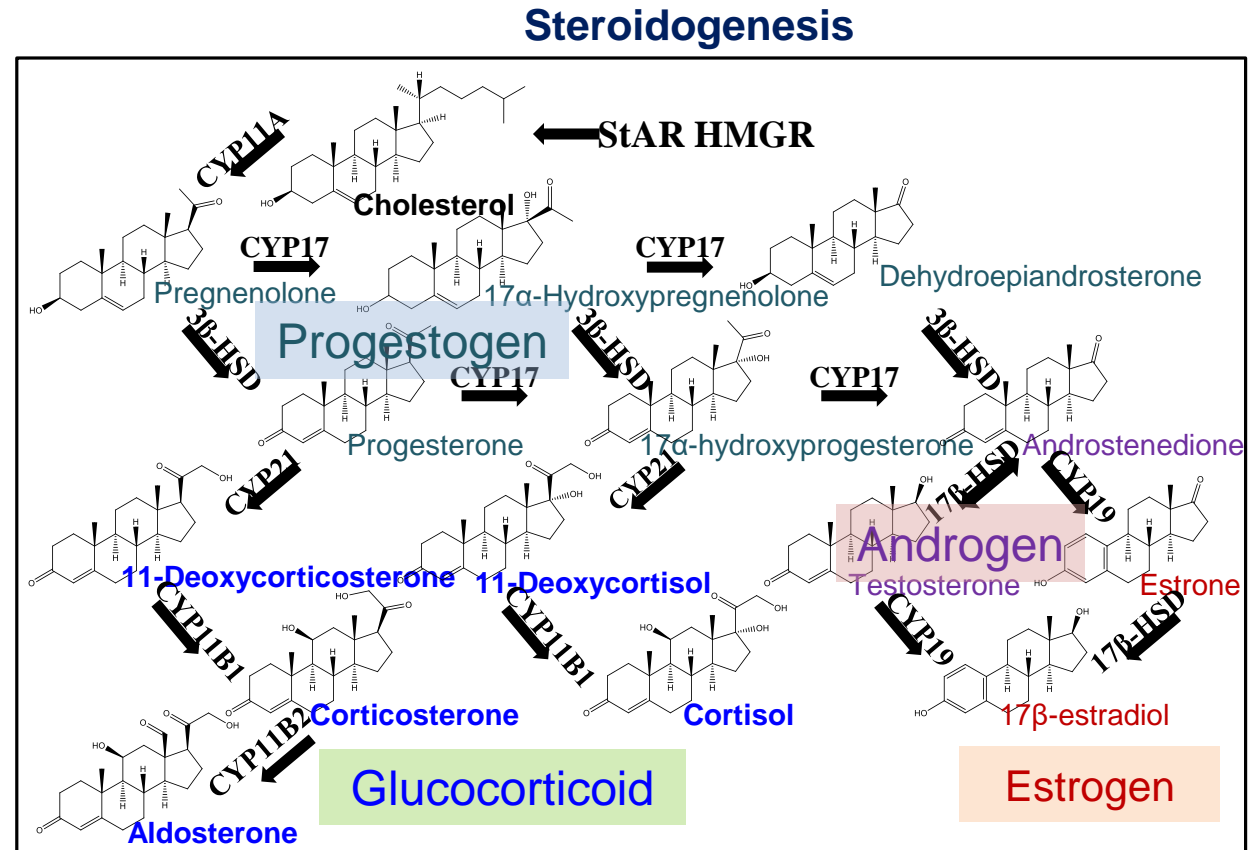
- Trace organic contaminants (TOrCs) occur in wastewater ubiquitously.
- Conventional WWTP is not efficacious for the attenuation of TOrCs.
- *Pharmaceuticals*
- *Personal care products*
- *Steroid hormones*
- *Industrial chemicals*





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	

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



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

Tamsin J. Runnalls ^a, Luigi Margiotta-Casaluci ^a, Subramaniam Kugathas ^a & John P. Sumpter ^a

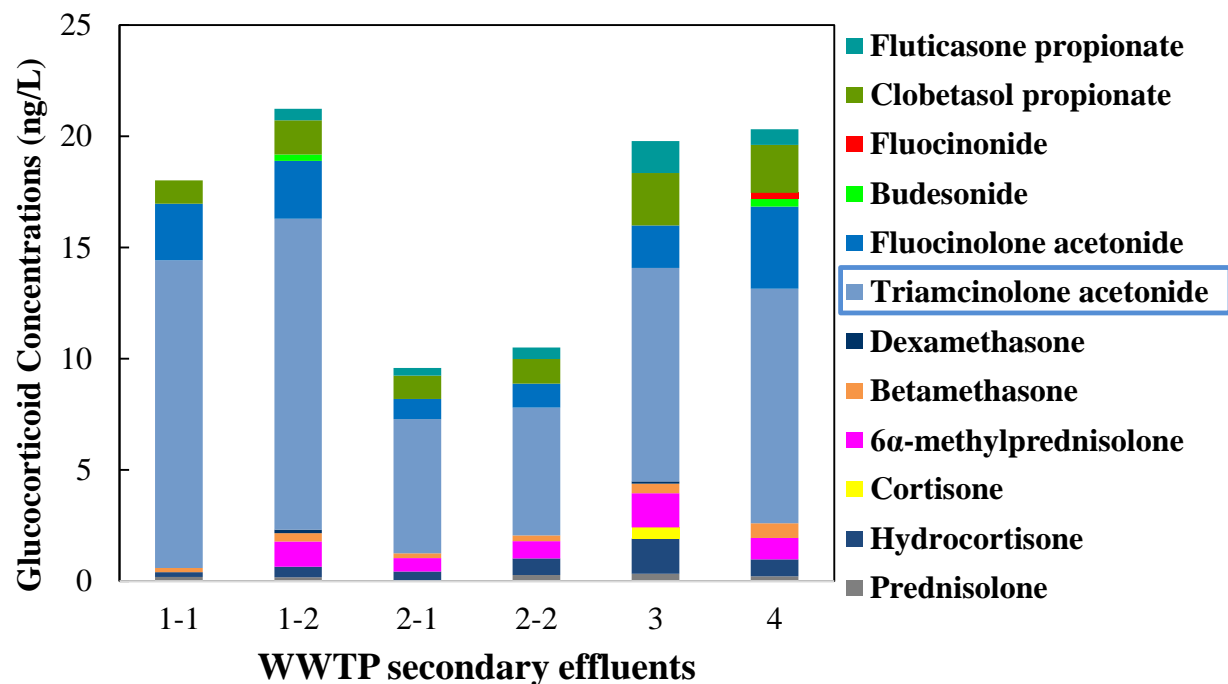
^a Institute for the Environment, Brunel University, Uxbridge, Middlesex, UK

Published online: 15 Dec 2010.

[Source: drug.com](http://www.drug.com) (database: IMS)



Glucocorticoids in WWTP effluents



49-77% of the total GCs

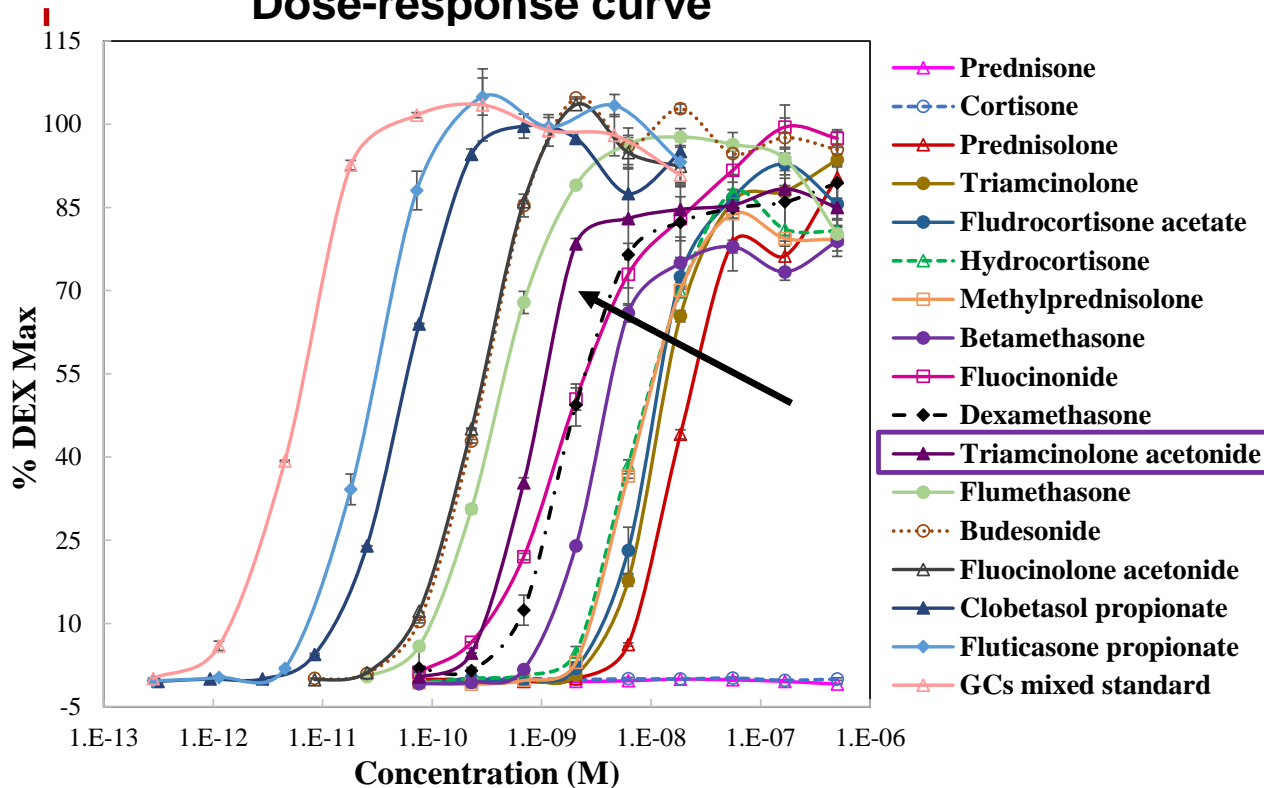
5.75 -14.0 ng/L

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



Relative potency of glucocorticoids

Dose-response curve



Most synthetic GCs have much higher activity than natural GCs

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



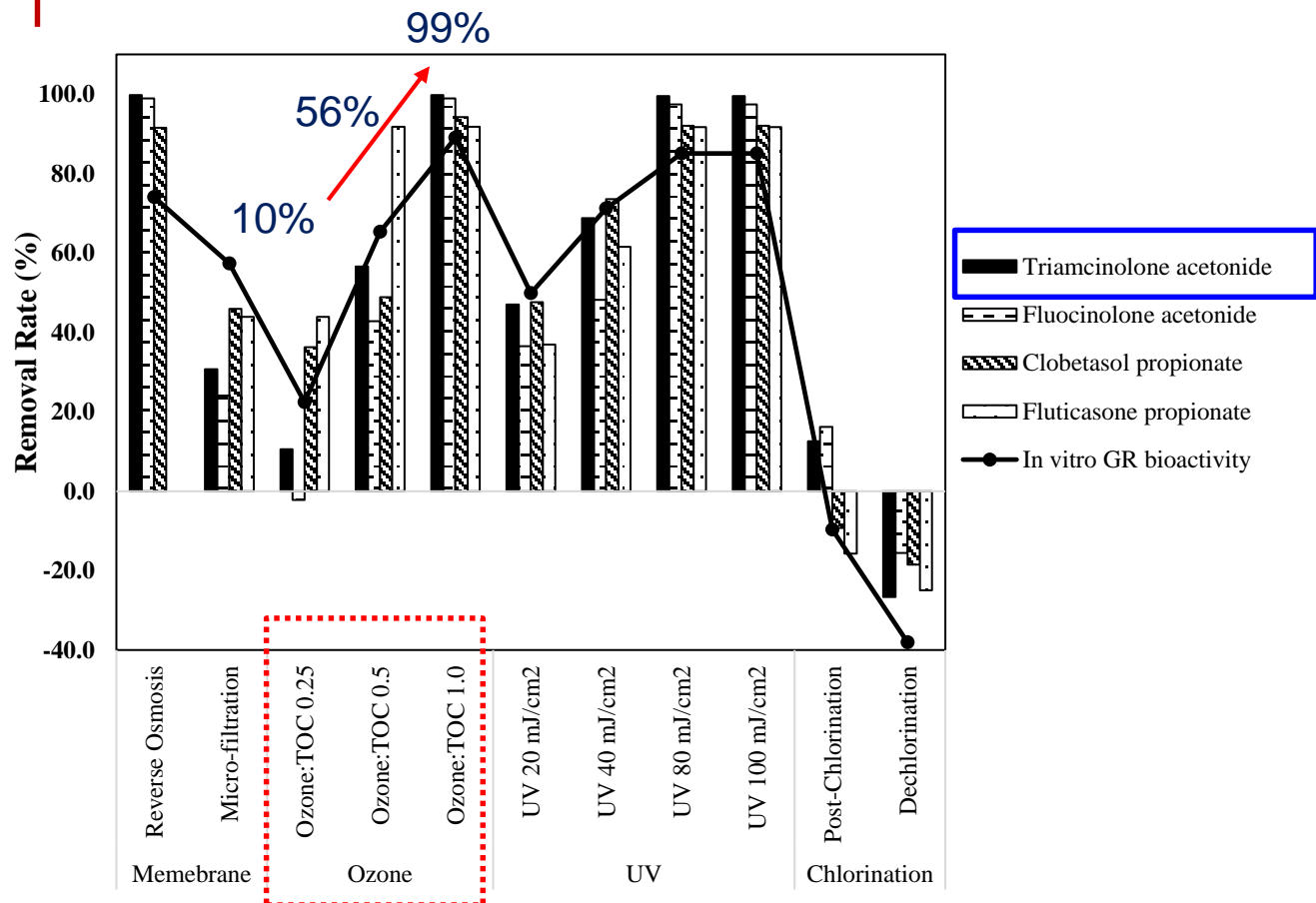
An increasing release of certain GCs is expected

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





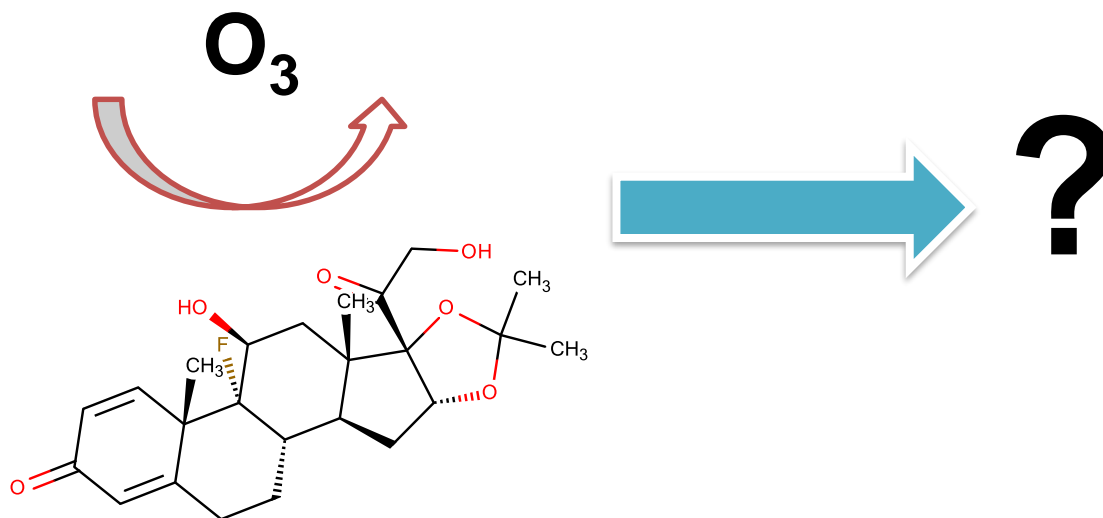
Attenuation of several GCs via advanced water treatment processes



- 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



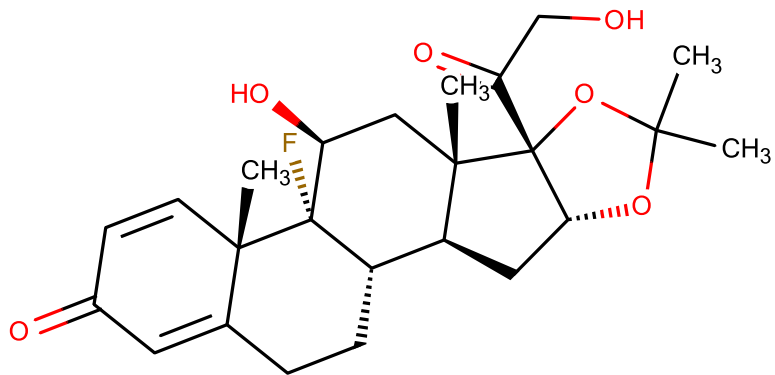
Transformation products





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



Formula: C₂₄H₃₁FO₆
Monoisotopic weight: 434.2104
Log *K*_{ow}: 2.53



Ozonation experiment

Generation of ozone stock solution



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

4 mM t-BuOH

< Xylem Wedeco 8HC modular >



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

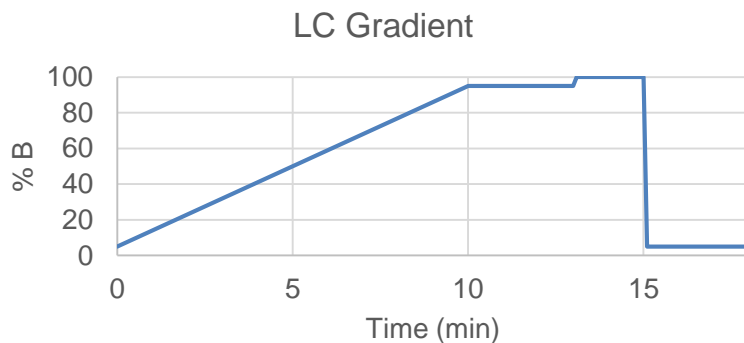




Instrumentation

LC run parameters

Column	Agilent Eclipse C18, 2.1 × 50 mm, 1.8 µm
Column Temp.	30 °C
Injection Volume	40 µL
Mobile Phase	H ₂ O-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.

The screenshot displays the TOF_POS_autoMSMS software interface. The 'Spectral Parameters' tab is active, showing a table of ion data. A red dashed circle highlights the 'Auto MS/MS Preferred/Exclude Table' section. The table lists ions with their precursor m/z, delta m/z, and other parameters. The 'On' column is checked for all entries. The 'Prec. m/z' column shows values like 297.1202, 295.1408, 304.0959, 391.0928, 391.0923, 391.0924, 366.0967, and 407.0966. The 'Delta m/z (ppm)' column shows values like 100, 100, 100, 100, 100, 100, 100, and 100. The 'Prec. Type' column shows 'Preferred' for all entries. The 'Ret. Time' column shows values like 8.851, 8.171, 4.771, 4.651, 8.006, 3.089, 2.811, and 2.911. The 'Is. Width' column shows 'Medium (~4 m/z)' for all entries. The 'Collision Energy' column is empty. The 'Default Values' section on the right shows 'Delta m/z: 100 ppm' and 'Delta Ret. Time: min'. The 'Use Preferred ion list only' checkbox is checked.

On	Prec. m/z	Delta m/z (ppm)	Z	Prec. Type	Ret. Time	Delta Ret. Time (min)	Is. Width	Collision Energy
<input checked="" type="checkbox"/>	297.1202	100	1	Preferred	8.851	1	Medium (~4 m/z)	
<input checked="" type="checkbox"/>	295.1408	100	1	Preferred	8.171	1	Medium (~4 m/z)	
<input checked="" type="checkbox"/>	304.0959	100	1	Preferred	4.771	1	Medium (~4 m/z)	
<input checked="" type="checkbox"/>	391.0928	100	1	Preferred	4.651	1	Medium (~4 m/z)	
<input checked="" type="checkbox"/>	391.0923	100	1	Preferred	8.006	1	Medium (~4 m/z)	
<input checked="" type="checkbox"/>	391.0924	100	1	Preferred	3.089	1	Medium (~4 m/z)	
<input checked="" type="checkbox"/>	366.0967	100	1	Preferred	2.811	1	Medium (~4 m/z)	
<input checked="" type="checkbox"/>	407.0966	100	1	Preferred	2.911	1	Medium (~4 m/z)	

< Preferred/Exclude Table >



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 PreO₃ and PostO₃

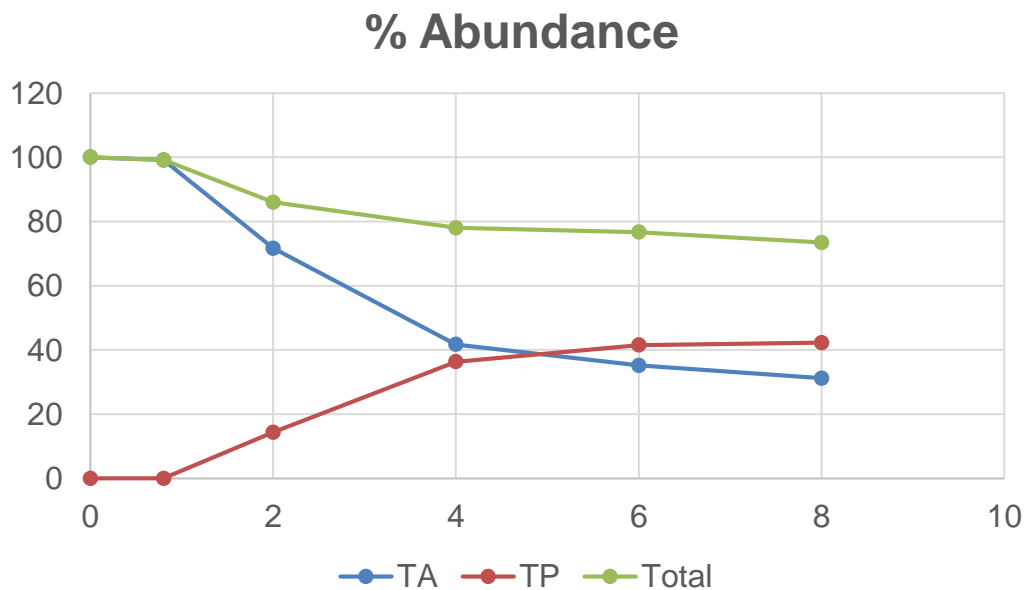
9

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



Profiling results

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





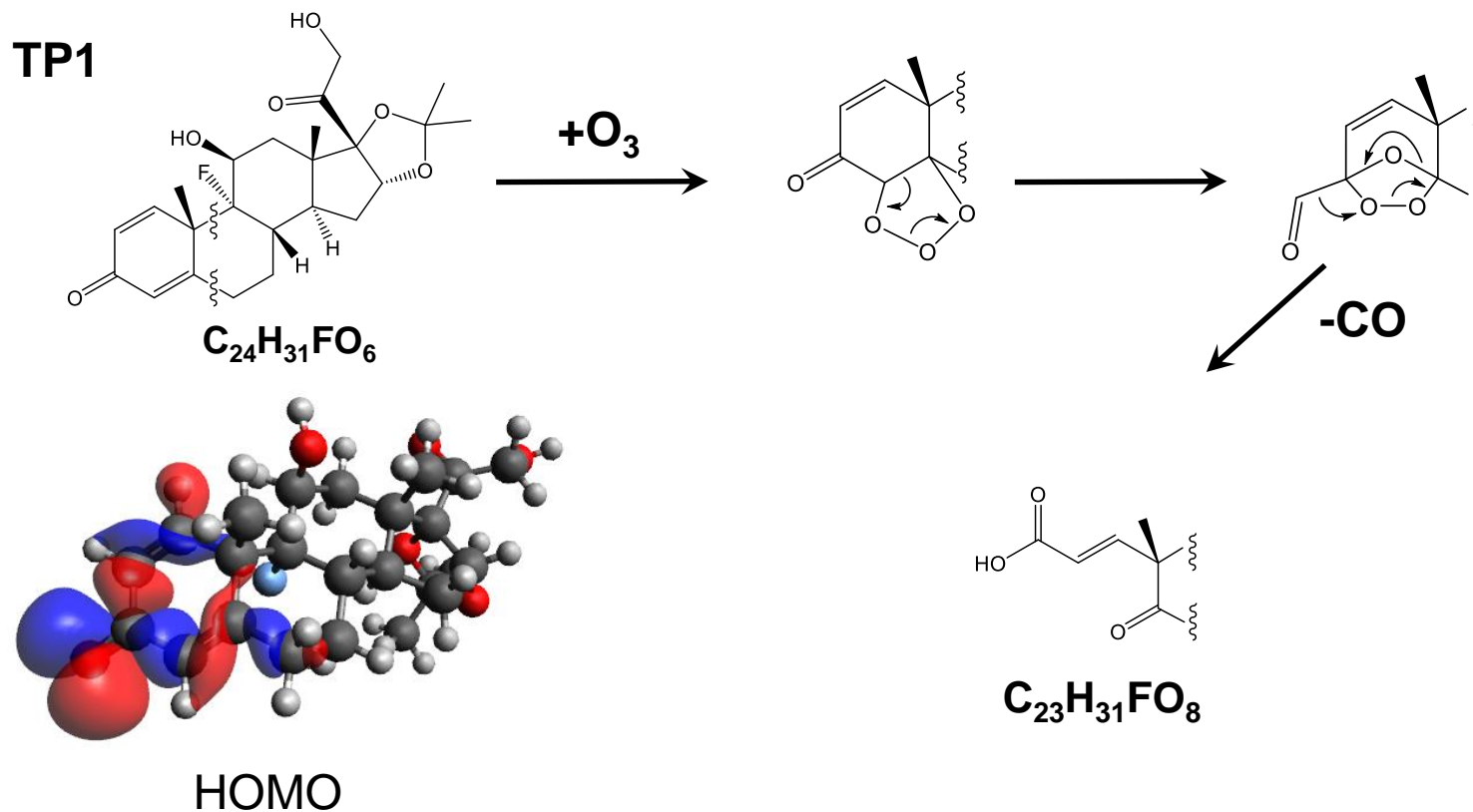
Profiling results

List of TPs

	Formula	Score	Mass	Diff (ppm)	RT
TP1	C₂₃ H₃₁ F O₈	95.7	454.2002	0.23	5.176
TP2	C ₂₃ H ₂₉ F O 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 F O 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 F O ₁₅	83.93	547.2063	-4.56	3.975
TP8	C ₃₀ H ₂₉ F O	81.9	424.2194	1.96	4.696
TP9	C ₂₉ H ₃₆ O ₁₀	85.19	544.2296	2.27	4.782

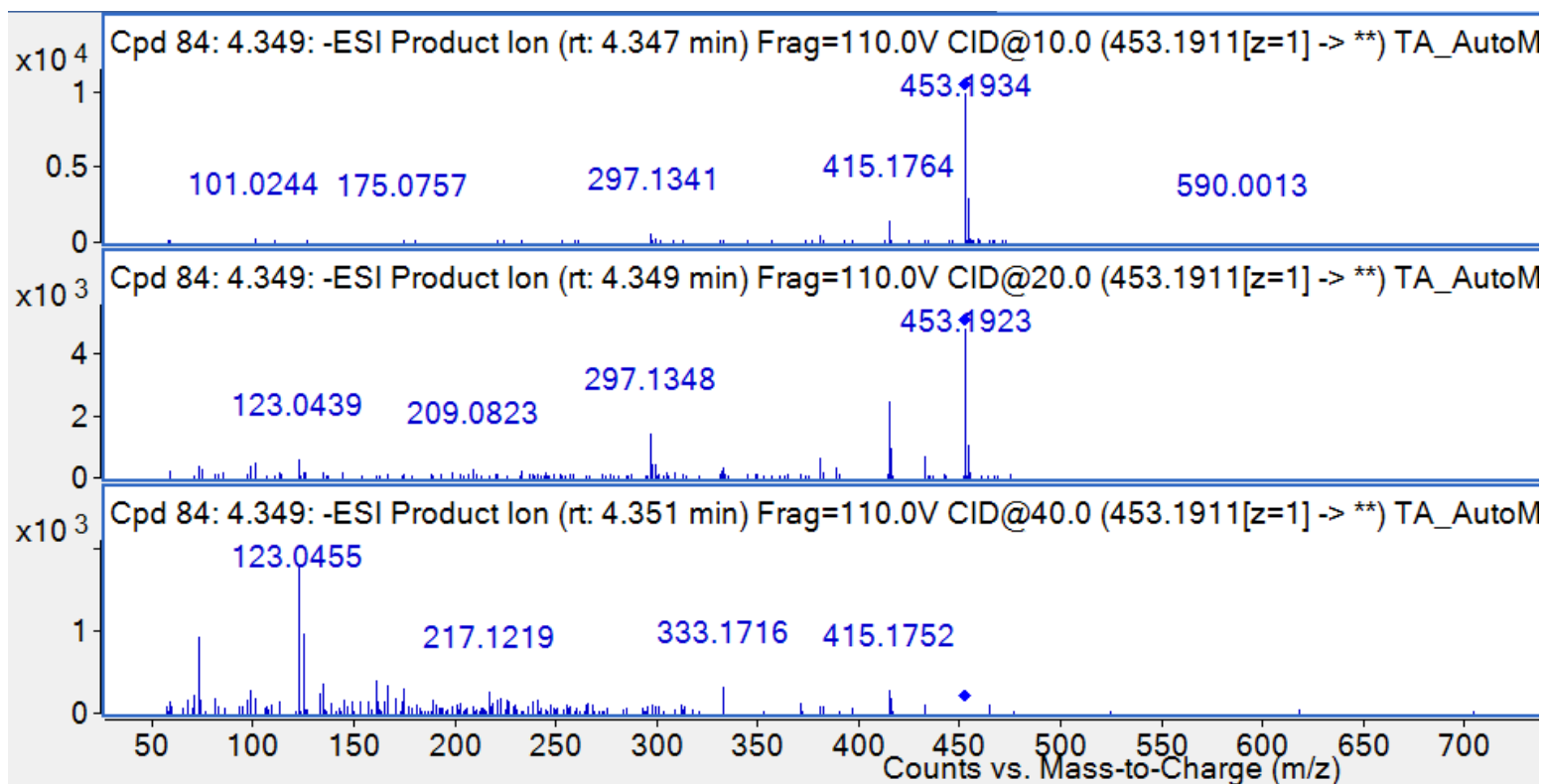


Proposed transformation pathway





Fragmentation pattern of TP1





MSC

Elucidated: 86.7% ions, 95.3% weights

Agilent MassHunter Molecular Structure Correlator 8.05.00 -- TA_t_AutoMS_C1_0_R1--M-H; ce=20

File Settings Help

Compound formula

M = 454.2003; 5 formula candidates from MFG

ID	Formula	Isomers	Taut. Gms	dM(ppm)	IdM(ppm)
1	C23H31FO8	3	1	-0.1	
2	C20H32F2O9	0	0	2.4	
3	C26H30O7	139	104	-2.6	
4	C17H33F3O10	0	0	5.0	
5	C24H29F3O5	23	16	-8.0	

Fragment formulas for C23H31FO8

m/z	intensity	formula	dM(ppm)
415.1769	2014.21	C20H28FO8	
415.1769	2014.21	C23H27O7	
297.1347	1475.68	C15H21O6	
297.1347	1475.68	C12H22FO7	
433.1880	749.03	C23H29O8	
333.1715	727.86	C16H26FO6	
333.1715	727.86	C19H25O5	
416.1810	516.88	C23H28O7	
416.1810	516.88	C20H29FO8	
123.0443	403.97	C7H7O2	
123.0443	403.97	C4H8FO3	
125.0618	388.67	C7H9O2	
381.1938	308.92	C17H30FO8	
381.1938	308.92	C20H29O7	
243.1039	298.33	C12H16FO4	
243.1039	298.33	C15H15O3	
331.1553	236.79	C19H23O5	
331.1553	236.79	C16H24FO6	
298.1371	227.43	C19H19FO2	
135.0438	225.43	C8H7O2	
135.0438	225.43	C5H8FO3	
389.1980	219.45	C19H30FO7	

Structure Search

Parameters: 0/0

ChemSpider (Web) Go Sort

Compound formula: C23H31FO8

Fragments of structure #1 - elucidated: 86.7% ions, 95.3% Weight

Mass	Intensity	Weight(%)	No. of candid.	Best score
415.1769	2014.21	40.3	16	93.3
297.1347	1475.68	4.0	0	0.0
433.1880	749.03	19.4	1	95.6
333.1715	727.86	3.9	23	95.4
416.1810	516.88	10.5	7	79.2
123.0443	403.97	0.0	9	88.7
125.0618	388.67	0.0	9	84.4
381.1938	308.92	3.7	9	80.4
243.1039	298.33	0.2	8	91.1
331.1553	236.79	1.2	21	95.5

Penalty=4.0 dM=-1.6ppm Score=93.3 C23H32O7-5H

Penalty=4.0 dM=-1.6ppm Score=93.3 C23H32O7-5H

Penalty=4.0 dM=-1.6ppm Score=93.3 C23H32O7-5H

Penalty=7.5 dM=1.1ppm Score=80.8 C20H29FO8-H

TP1

Standard InChIKey: GHBPWCMRWBPBOKQ-FYAYRADVSA-N

Compatibility Score: 83.17

MSC Save Delete

Tentative fragmentation pattern

Compatibility Score: 83.17



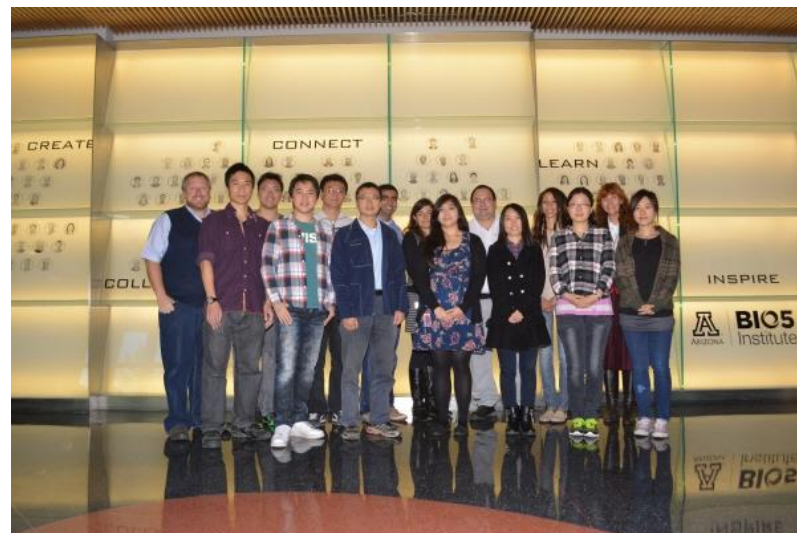
Conclusion

- 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.



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Agilent Technologies

WEDECO
a xylem brand

NEMC



Q&A



Questions???

