ELECTRONIC SUPPORTING INFORMATION

Formation of Bioactive Transformation Products during Glucocorticoid Chlorination

Nicholas C. Pflug,[1] Allison Kupsco,[2] Edward P. Kolodziej,[3,4] Daniel Schlenk,[2] Lynn M. Teesch,[5] James B. Gloer,[1] David M. Cwiertny[6]

[1]*Department of Chemistry, University of Iowa; Iowa City, IA 52242*

[2]*Department of Environmental Sciences, University of California, Riverside; Riverside, CA 92521*

[3]*Department of Interdisciplinary Arts and Sciences, University of Washington; Seattle, WA 98195*

[4]*Department of Civil and Environmental Engineering, University of Washington; Seattle, WA 98195*

[5]*High Resolution Mass Spectrometry Facility, University of Iowa; Iowa City, IA 52242*

[6]*Department of Civil & Environmental Engineering, University of Iowa; Iowa City, IA 52242*

*Corresponding Authors:

David M. Cwiertny
Email: david-cwiertny@uiowa.edu; Phone: 319-335-1401
Department of Civil and Environmental Engineering
4136 Seamans Center
Iowa City, IA 52242

James B. Gloer
Email: james-gloer@uiowa.edu; Phone: 319-335-1361
Department of Chemistry
E331 Chemistry Building
Iowa City, IA 52242

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Contents: 2 Tables, 32 Figures, 32 Pages

TABLES

Table S1: HRESITOFMS results of GC chlorination reaction mixtures.

Parent	Products	Retention Time	Calculated (M+H)	Observed (M+H)	Standard (M+H)	Notes
prednisone		7.34	359.1858	359.1857	359.1863	
	hydroxyprednisone	7.88	375.1807	375.1801		
	9-chloro-prednisone	8.34	393.1469	393.1476		confirmed by ^{1}H NMR and HMBC
	d1-adrenosterone	9.99	299.1647	299.1649	299.1629	confirmed by ^{1}H NMR
	chlorinated d1-adrenosterone	11.8	333.1257	333.1259		
prednisolone		7.58	361.2015	361.2013	361.2019	
	prednisone	7.79	359.1858	359.1857	359.1863	
	chlorinated prednisone	8.53	393.1469	393.1461		
	chlorinated prednisolone	9.30	395.1625	395.1615		
	11β-hydroxyboldione	9.53	301.1803	301.1816		
	d1-adrenosterone	9.84	299.1647	299.1636	299.1629	
	chlorinated d1-adrenosterone	11.05	333.1257	333.1281		
cortisone		6.81	361.2015	361.1953	361.1983	
	prednisone	6.56	359.1858	359.1841	359.1863	
	chlorinated prednisone	7.23	393.1469	393.1407		
	chlorinated prednisone	7.57	393.1469	393.1407		
	d1-adrenosterone	9.12	299.1647	299.1632	299.1629	
	adrenosterone	9.58	301.1803	301.1796	301.1798	
cortisol		7.32	363.2171	363.2148	363.2152	
	prednisolone	7.05	361.2015	361.1996	361.2019	confirmed by ^{1}H NMR
	cortisone	7.59	361.2015	361.2019	361.1983	
	chlorinated prednisolone	8.23	395.1625	395.1611		
	chlorinated prednisolone	9.35	395.1625	395.1610		
dexamethasone		8.89	393.2077	393.2066	393.2055	
	11-keto-dexamethasone	9.28	391.1921	391.1910		
	17-oxo-dexamethasone	11.52	333.1866	333.1858		

Table S2: NMR data of 9-chloro-prednisone in $CDCl_3$ (600 MHz).

Proton	δ_H mult. (*J* in Hz)	HMBC (H# → C#)	Carbon	δ_C
1	7.63 d (10.4)	3, 5, 9, 10, 19	1	152.7
2	6.26 dd (10.4, 1.8)	4, 10	2	128.9
4	6.15 t (1.8)	2, 6, 10	3	186.2
6	2.44 m	4, 5, 7	4	127.3
	2.58 td (13.7, 5.7)		5	162.2
7	1.82 m	5, 6, 8, 9	6	31.5
	1.89 m		7	26.1
8	2.46 m	7, 9, 14	8	38.8
12	2.08 d (12.8)	9, 11, 13, 14, 17, 18	9	78.0
	3.64 d (12.8)		10	48.6
14	2.82 m	8, 9, 13, 15, 18	11	202.7
15	1.52 m	8, 13, 14, 16, 17	12	45.1
	1.93 m		13	50.9
16	1.74 ddd (14.9, 9.5, 5.8)	13, 14, 15, 17, 20	14	43.3
	2.77 m		15	22.7
18	0.71 s	12, 13, 14, 17	16	34.9
19	1.58 s	1, 5, 9, 10	17	87.9
21	4.27 d (19.7)	20	18	15.8
	4.65 d (19.7)		19	21.6
21-OH	2.99 br s		20	210.1
			21	67.5

FIGURES

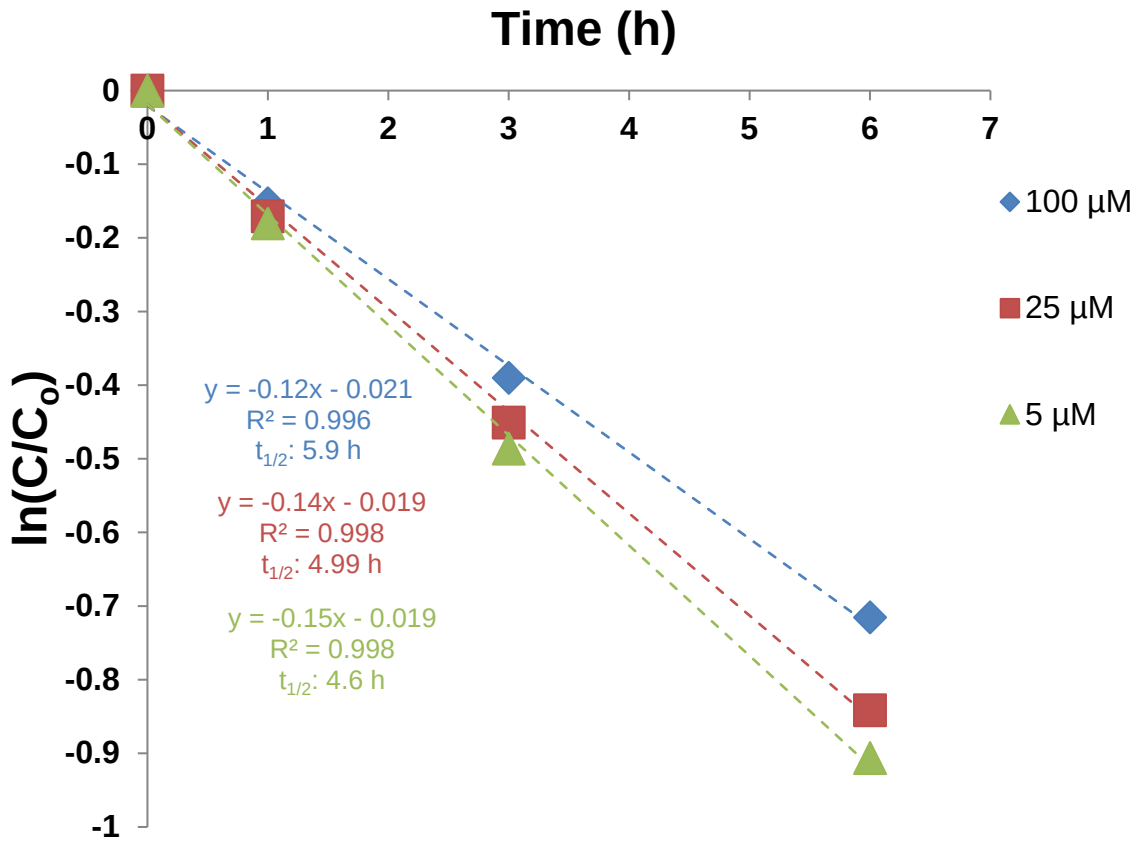
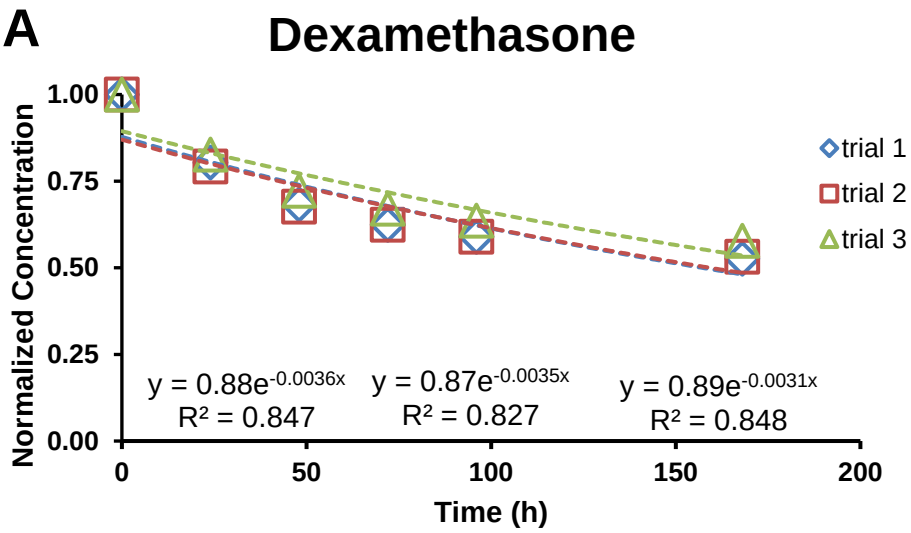
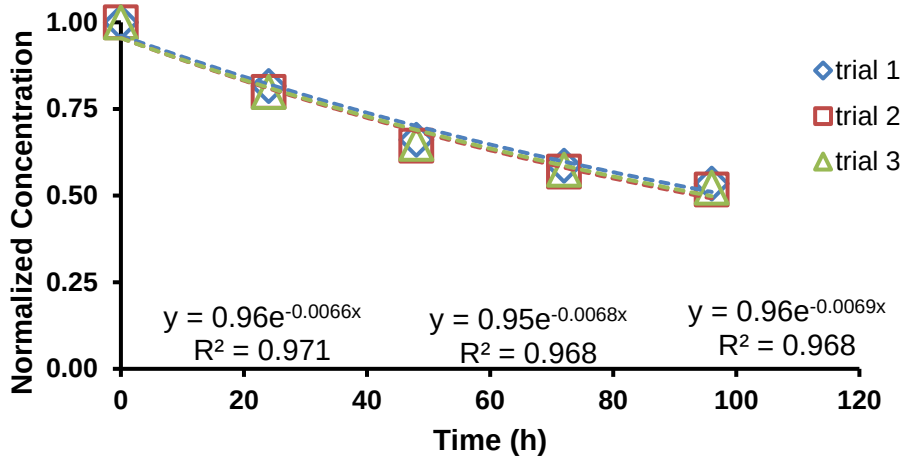


Figure S1: Semi-log plot of normalized concentration versus time as a function of initial cortisol concentration. Rate coefficients for GC chlorination were quantified using excess chlorine concentrations that allowed all GC decay to be approximated as pseudo-first order (i.e., following exponential decay). Here, at 100 mg/L Cl_2/L, systems with initial cortisol concentrations from 5 - 100 µM (~ 2 - 40 mg/L; GC:Cl_2 molar ratios ranged from ~ 1:14 to 1:280 mol), exhibited half-lives of ~ 5 hours. This behavior is consistent with sufficient excess of HOCl such that its concentration can reasonably be assumed as constant over the course of these reactions.

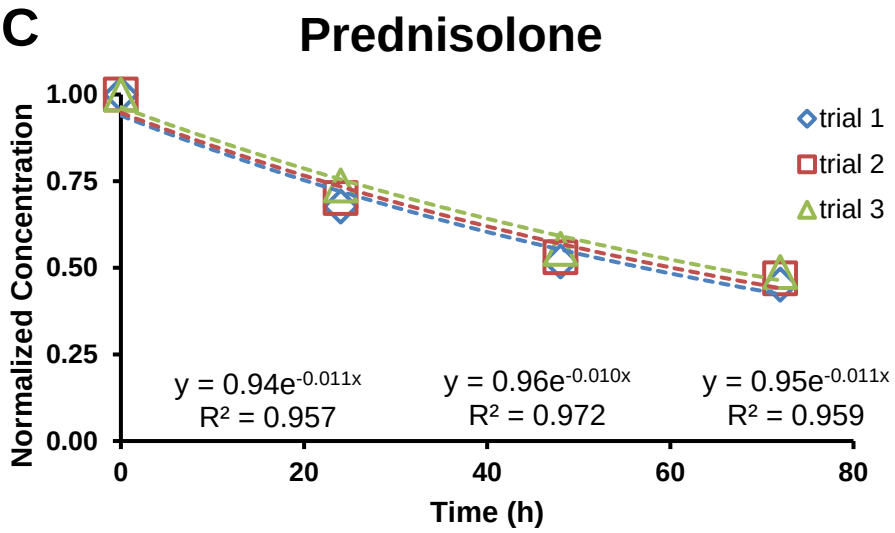
A


Dexamethasone
Normalized Concentration
1.00
0.75
0.50
0.25
0.00
0
50
100
150
200
Time (h)
trial 1
trial 2
trial 3
y = 0.88e-0.0036x
R² = 0.847
y = 0.87e-0.0035x
R² = 0.827
y = 0.89e-0.0031x
R² = 0.848

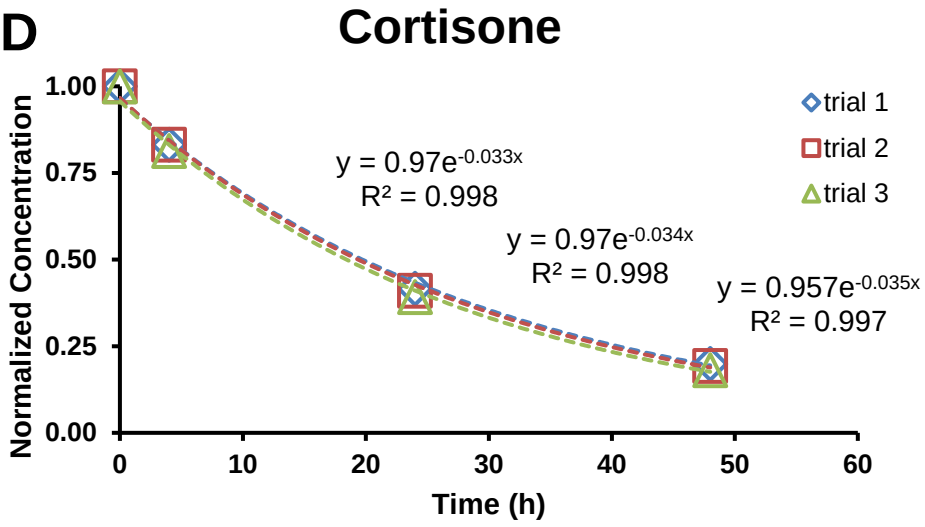
B


Prednisone
Normalized Concentration
1.00
0.75
0.50
0.25
0.00
0
20
40
60
80
100
120
Time (h)
trial 1
trial 2
trial 3
y = 0.96e-0.0066x
R² = 0.971
y = 0.95e-0.0068x
R² = 0.968
y = 0.96e-0.0069x
R² = 0.968

C


Prednisolone
Normalized Concentration
1.00
0.75
0.50
0.25
0.00
0
20
40
60
80
Time (h)
trial 1
trial 2
trial 3
y = 0.94e-0.011x
R² = 0.957
y = 0.96e-0.010x
R² = 0.972
y = 0.95e-0.011x
R² = 0.959

D


Cortisone
Normalized Concentration
1.00
0.75
0.50
0.25
0.00
0
10
20
30
40
50
60
Time (h)
trial 1
trial 2
trial 3
y = 0.97e-0.033x
R² = 0.998
y = 0.97e-0.034x
R² = 0.998
y = 0.957e-0.035x
R² = 0.997

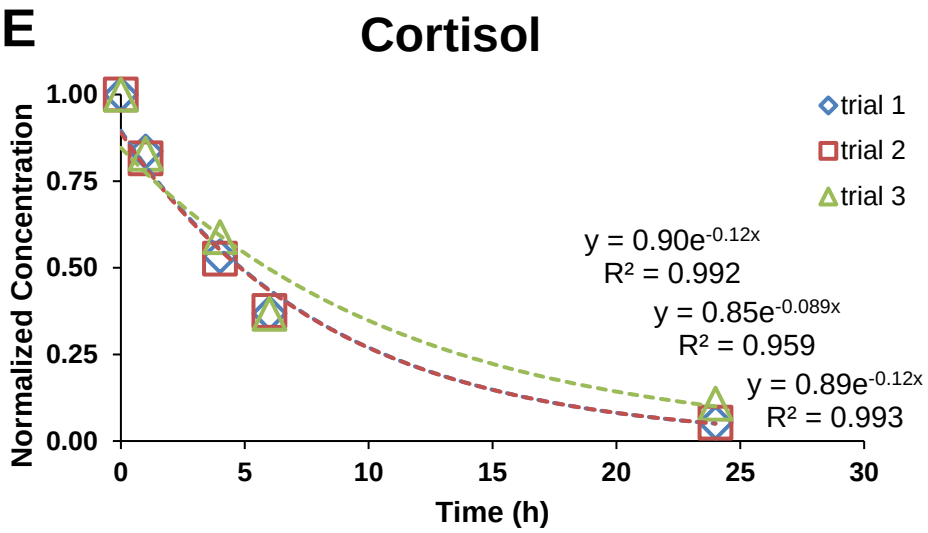


Figure S2: Normalized GC concentration over time during reaction with free chlorine for (A) dexamethasone, (B) prednisone, (C) prednisolone, (D) cortisone, and (E) cortisol. Reaction conditions: Initial GC concentration of 10 µM, initial chlorine concentration of 100 mg Cl_2/L, and pH 7. Exponential decay model fits are shown as dashed lines for replicate (n = 3) trials.

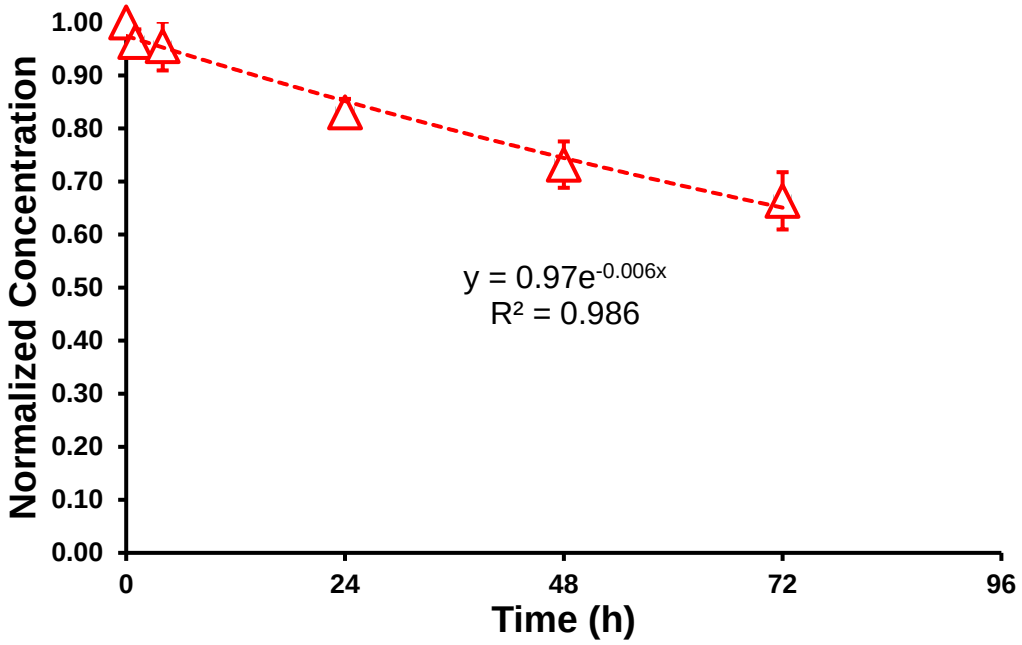


Figure S3: Normalized concentration of cortisol over time during reaction with free chlorine using experimental conditions representative of water or wastewater treatment. Reaction conditions: Initial cortisol concentration of 50 µg/L, initial chlorine concentration of 5 mg Cl_2/L, and pH 7. Error bars represent standard deviation of n = 3 replicates.

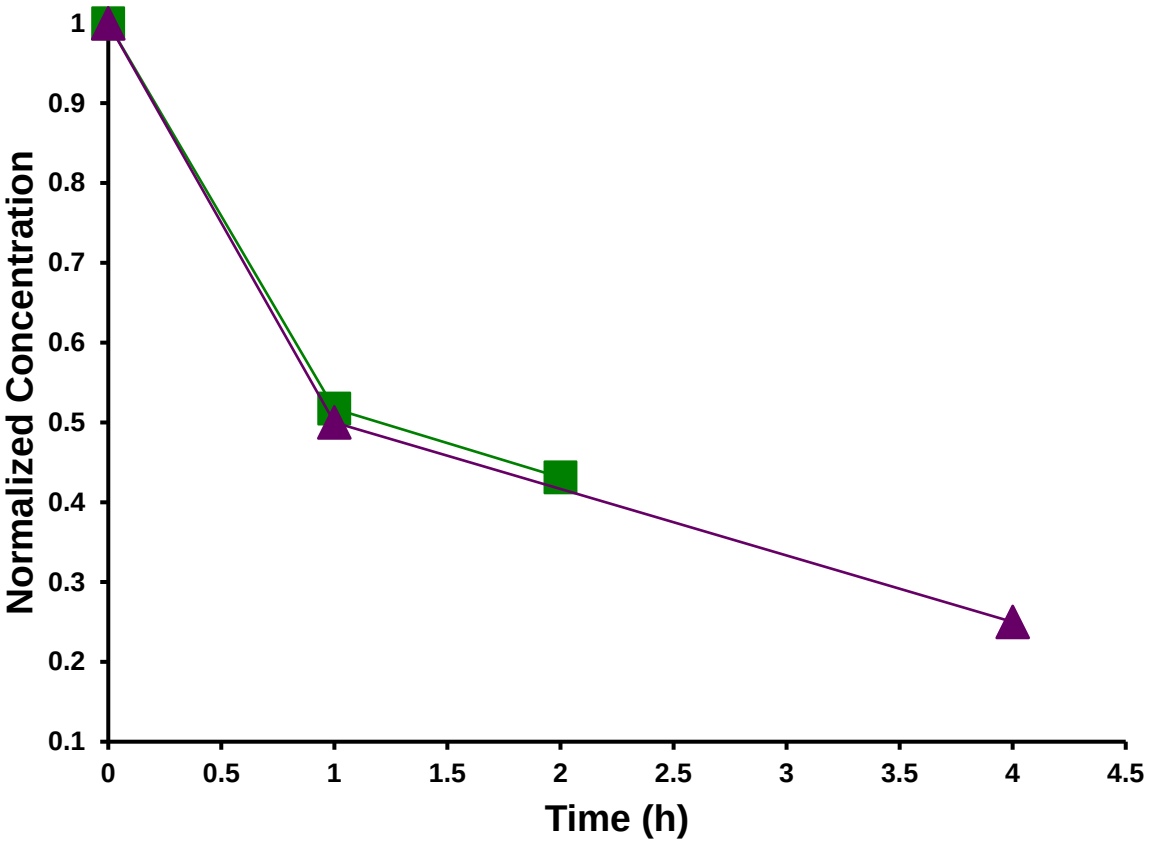


Figure S4: Concentration of 17β-trenbolone (green squares) and estrone (purple triangles) over time during reaction with free chlorine. Reaction conditions: Initial steroid concentration of 25 μM, initial chlorine concentration of 2.5 mg Cl_2/L, and pH 7.

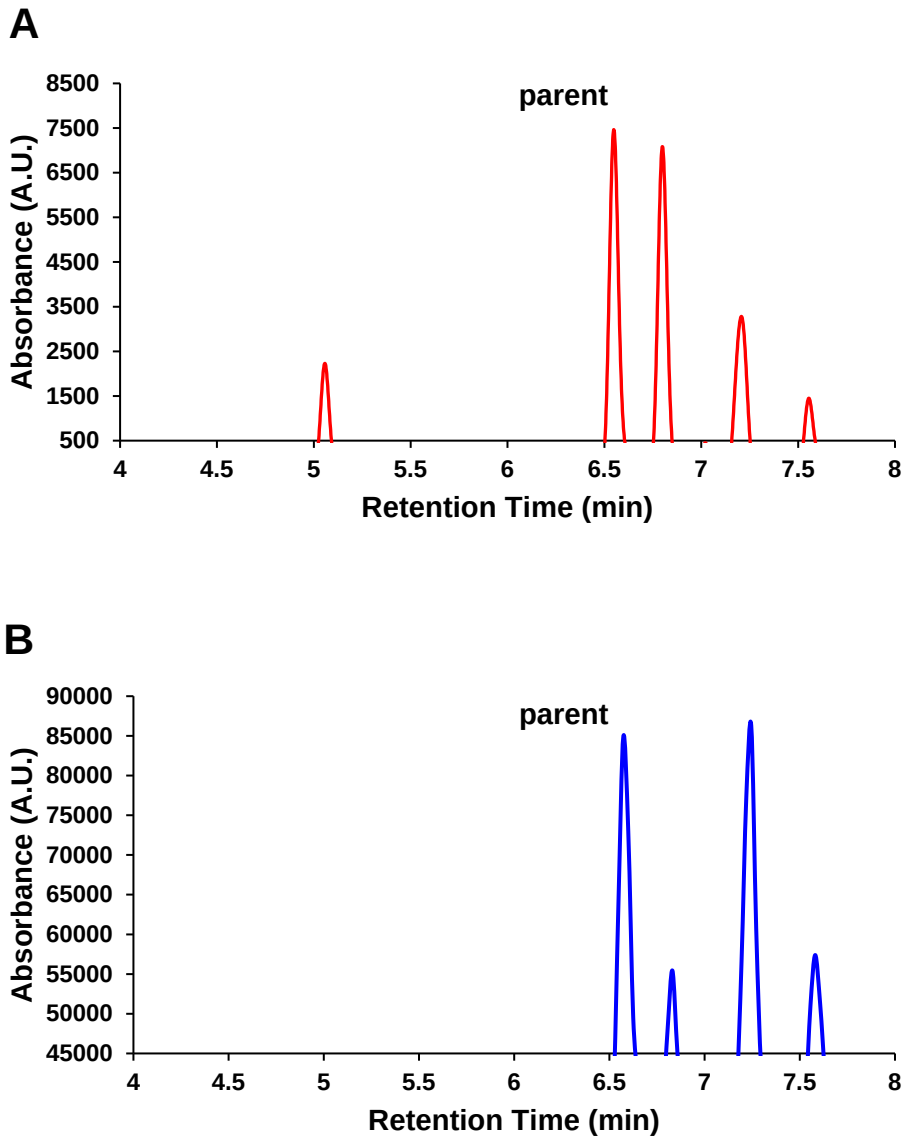


Figure S5: Trace of LC chromatograms with (A) UV detection (λ_{max} 254 nm) and (B) MS detection for product mixtures (with residual parent) for prednisolone chlorination reaction. As illustrated here for prednisolone, and as was generally observed for most GC reaction mixtures, analysis of product mixtures revealed formation of multiple, more hydrophobic transformation products. In some instances, more polar products were observed by UV with diode array detection (DAD), although these more polar species did not readily ionize during repeated attempts to further characterize their nature via MS.

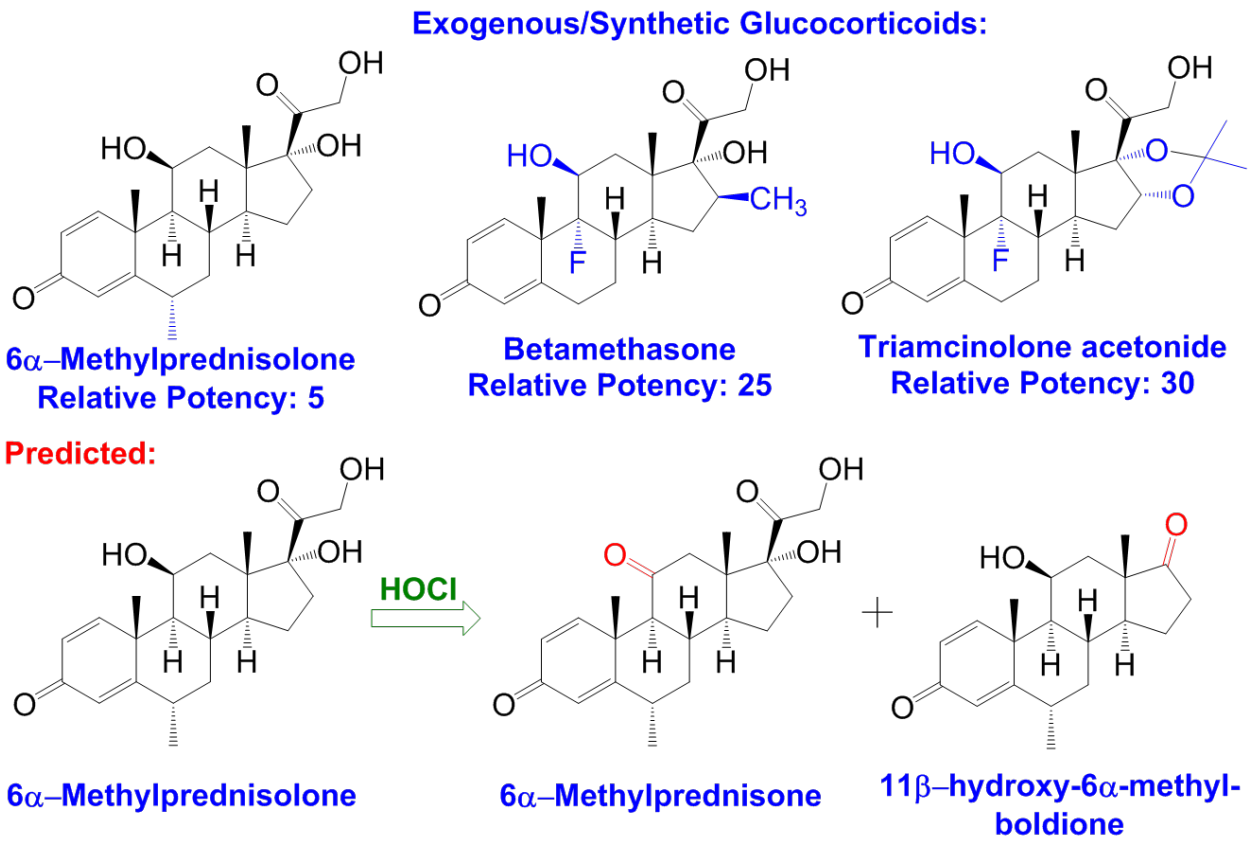


Figure S6: (top) Synthetic GCs and relative anti-inflammatory potencies and (bottom) predicted chlorination products based on common transformations unveiled in this study for GCs investigated.

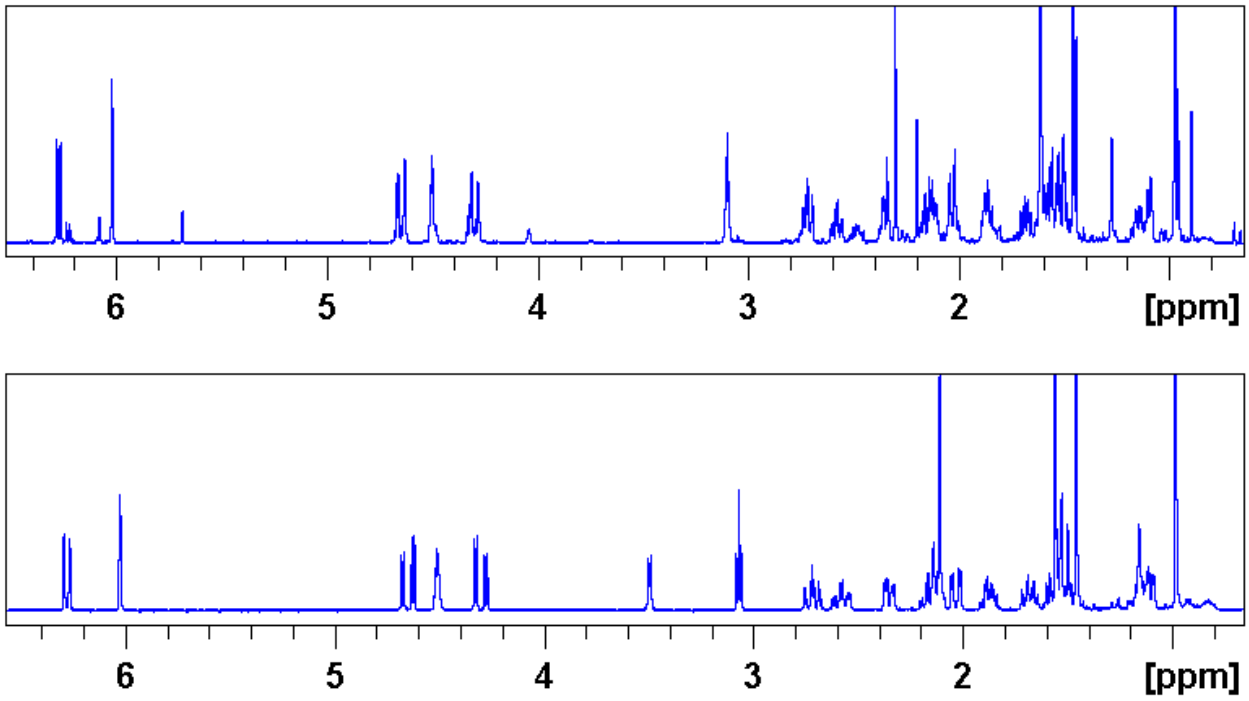


Figure S7: ^{1}H NMR spectrum of cortisol chlorination fraction 4 (top) and prednisolone standard (bottom) in $CDCl_3$ (600 MHz). Signal at 3.5 ppm in the spectrum of prednisolone standard corresponds to 11-OH.

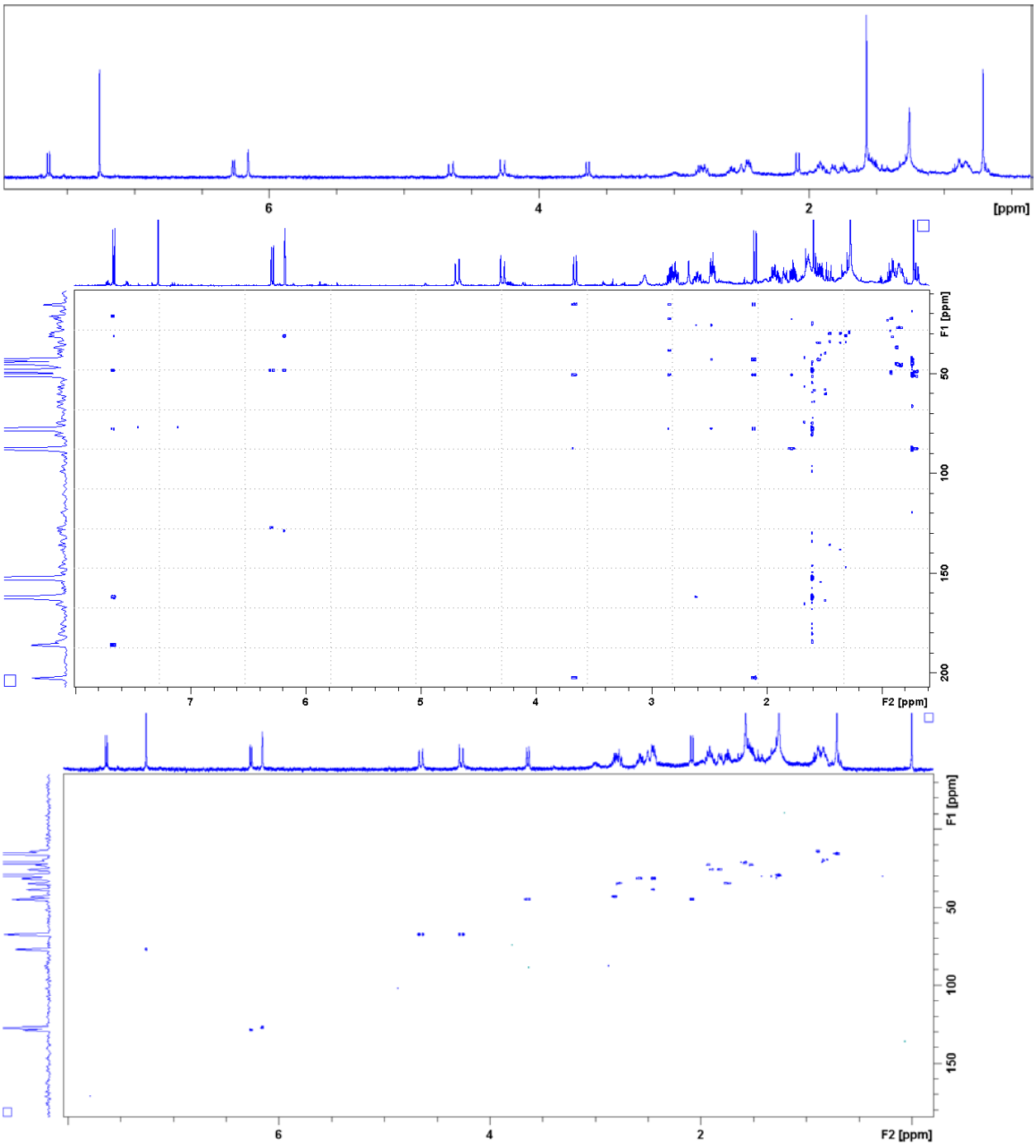


Figure S8: ^{1}H NMR (top), HMBC (middle), and HSQC (bottom) spectra of prednisone chlorination fraction 2 (9-chloro-prednisone) in $CDCl_3$ (600 MHz).

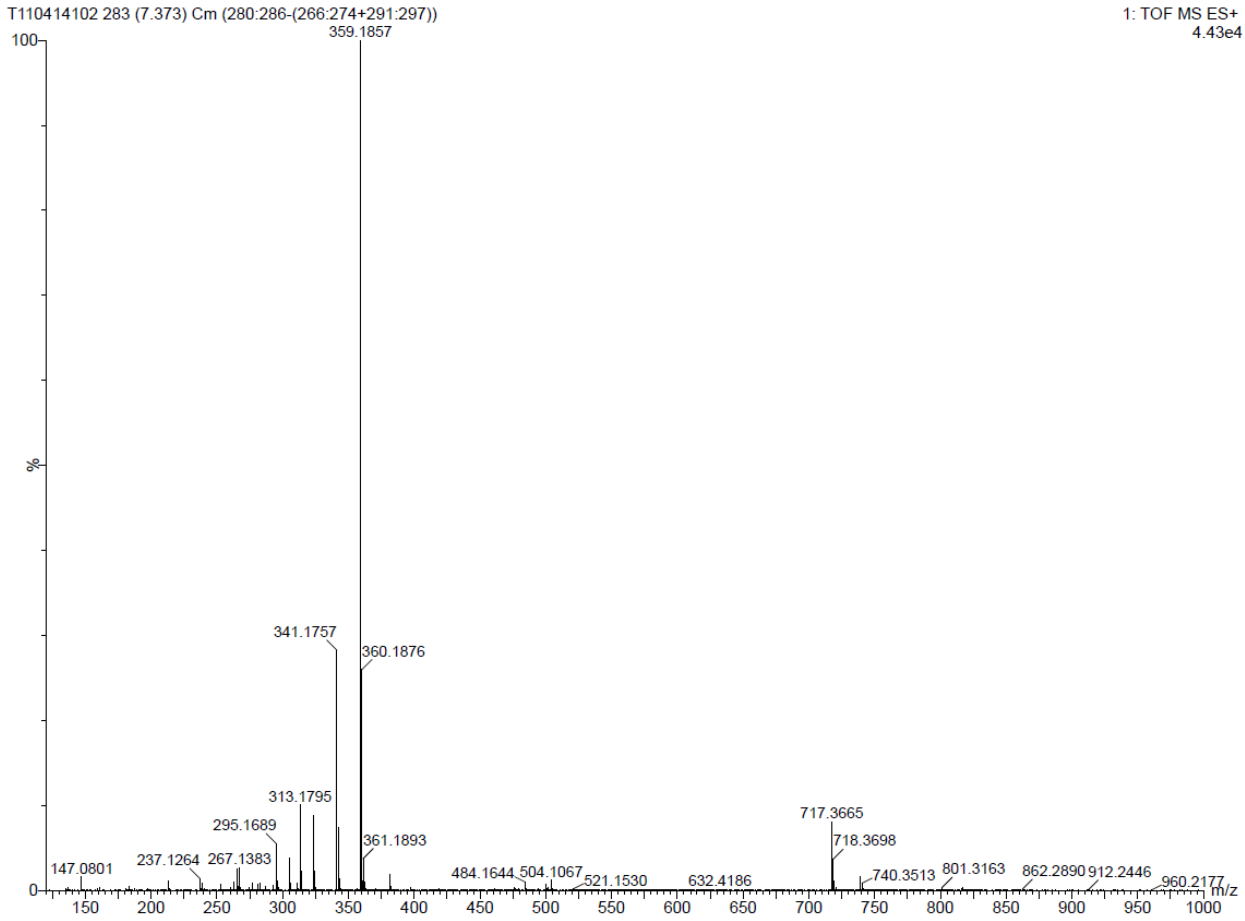


Figure S9: HRESITOFMS of prednisone standard.

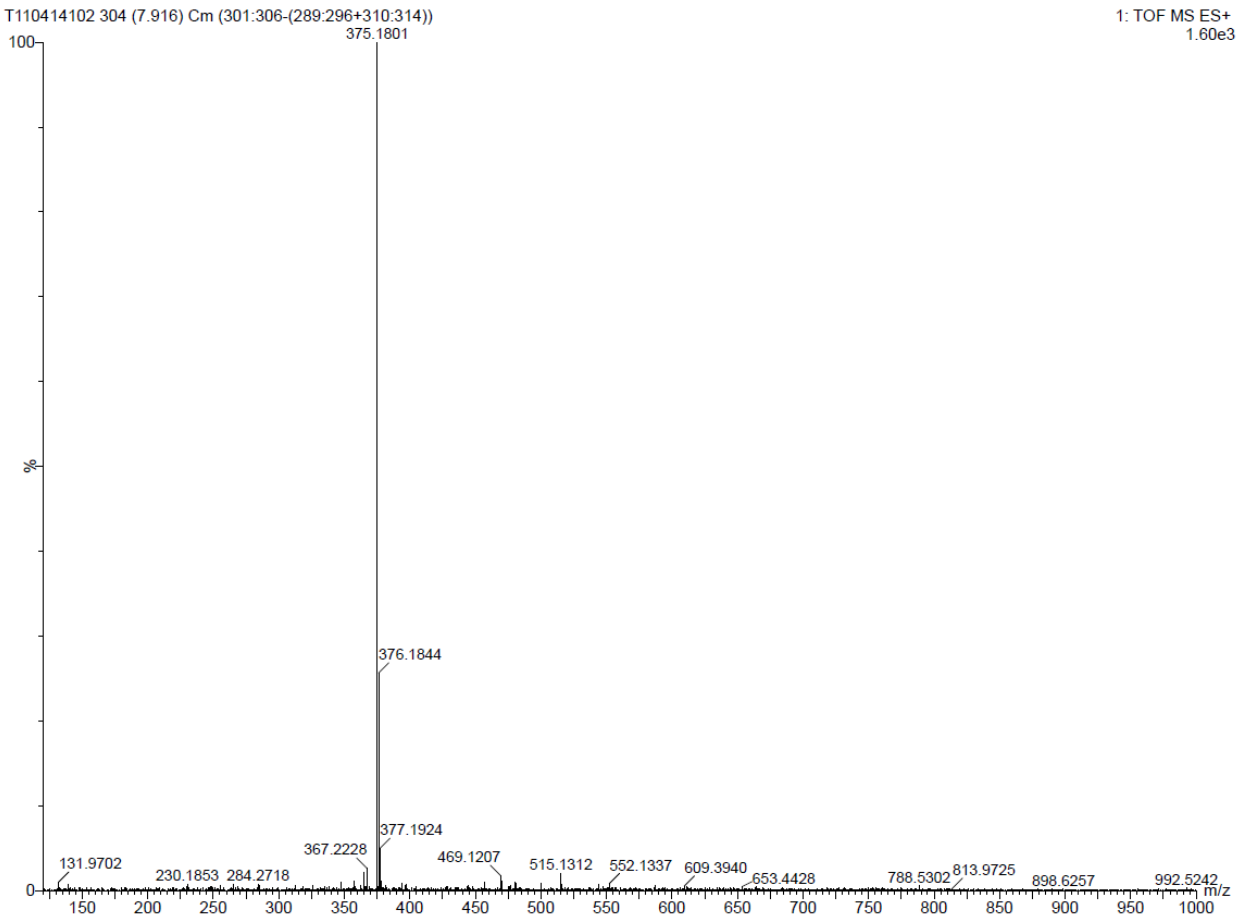


Figure S10: HRESITOFMS of hydroxyprednisone product resulting from the reaction of prednisone with free chlorine.

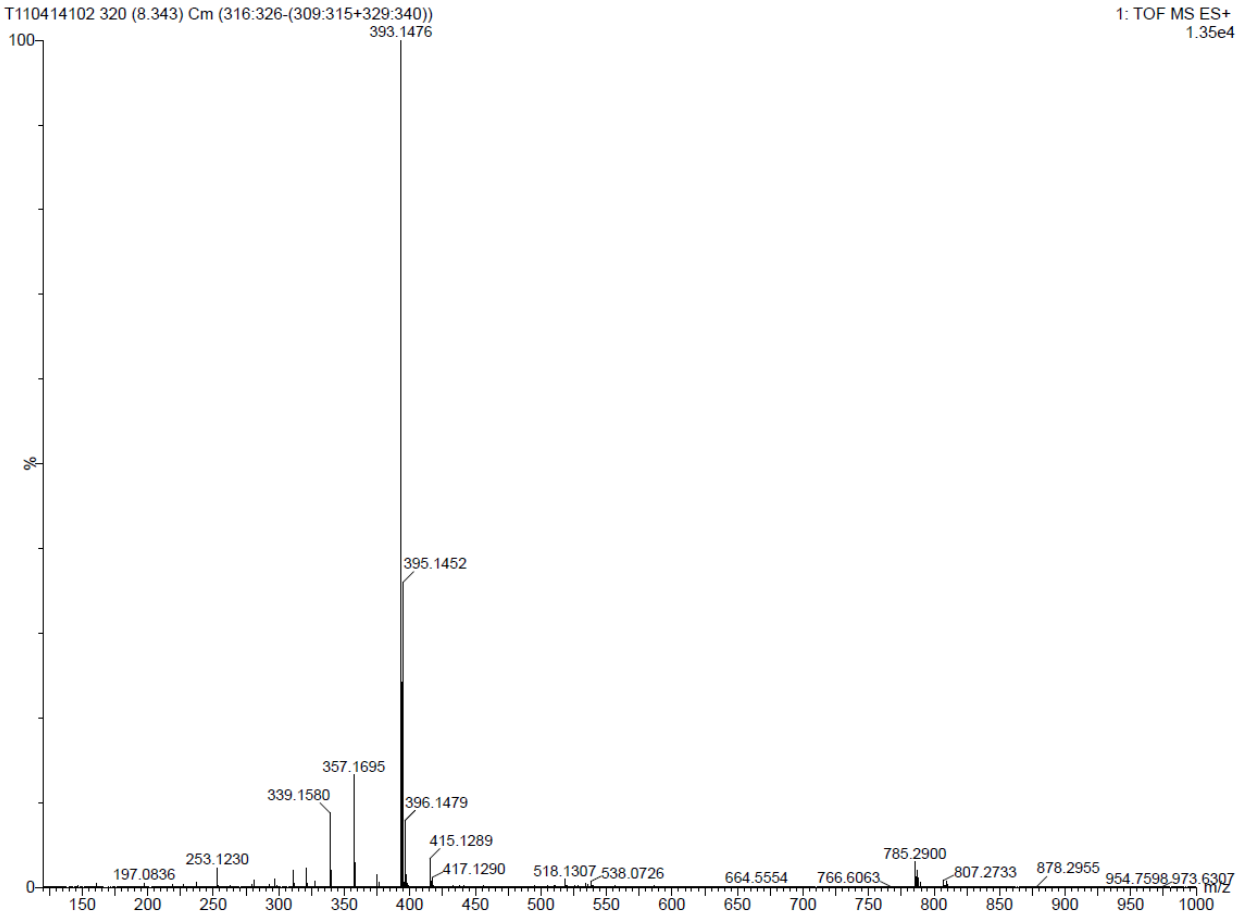


Figure S11: HRESITOFMS of 9-chloroprednisone product resulting from the reaction of prednisone with free chlorine.

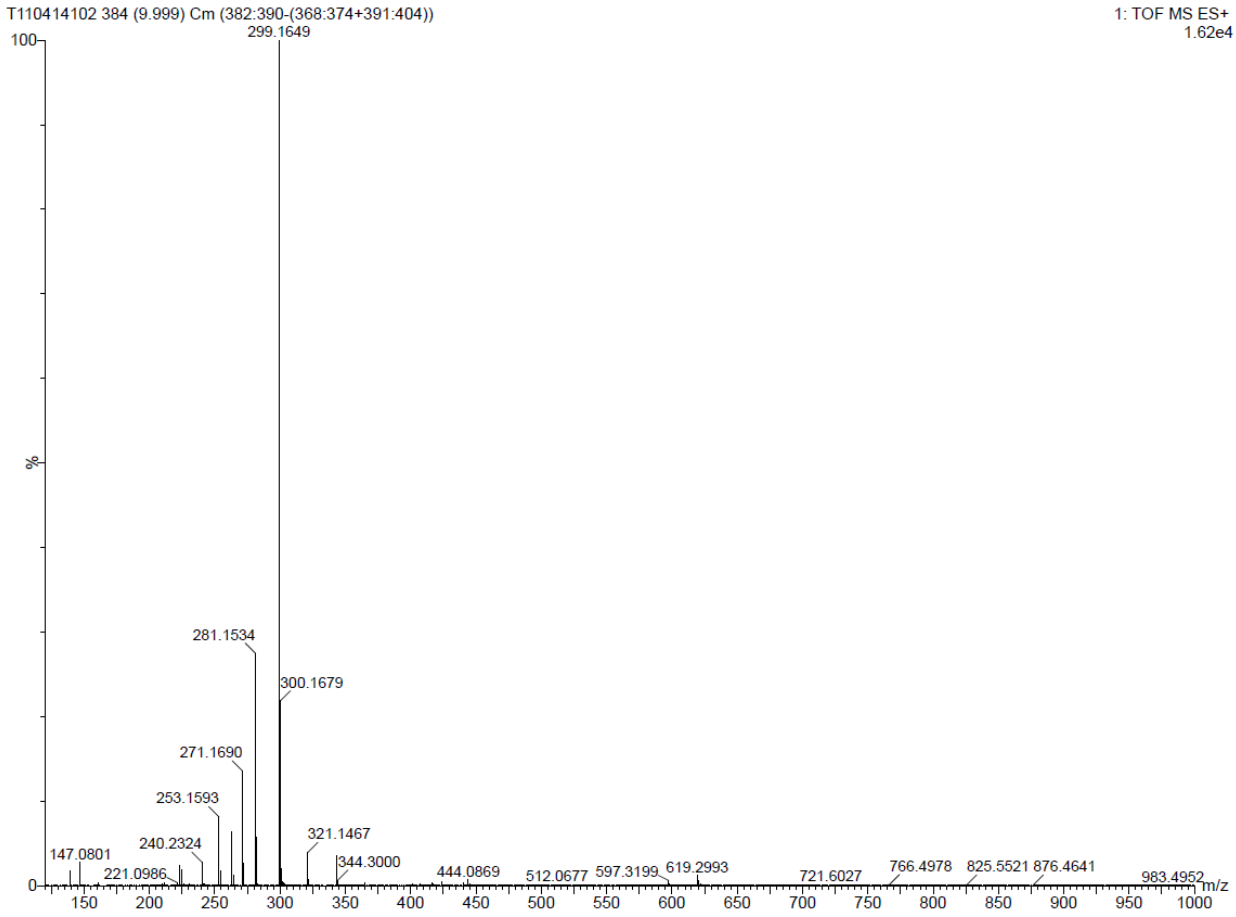


Figure S12: HRESITOFMS of d1-adrenosterone product resulting from the reaction of prednisone with free chlorine.

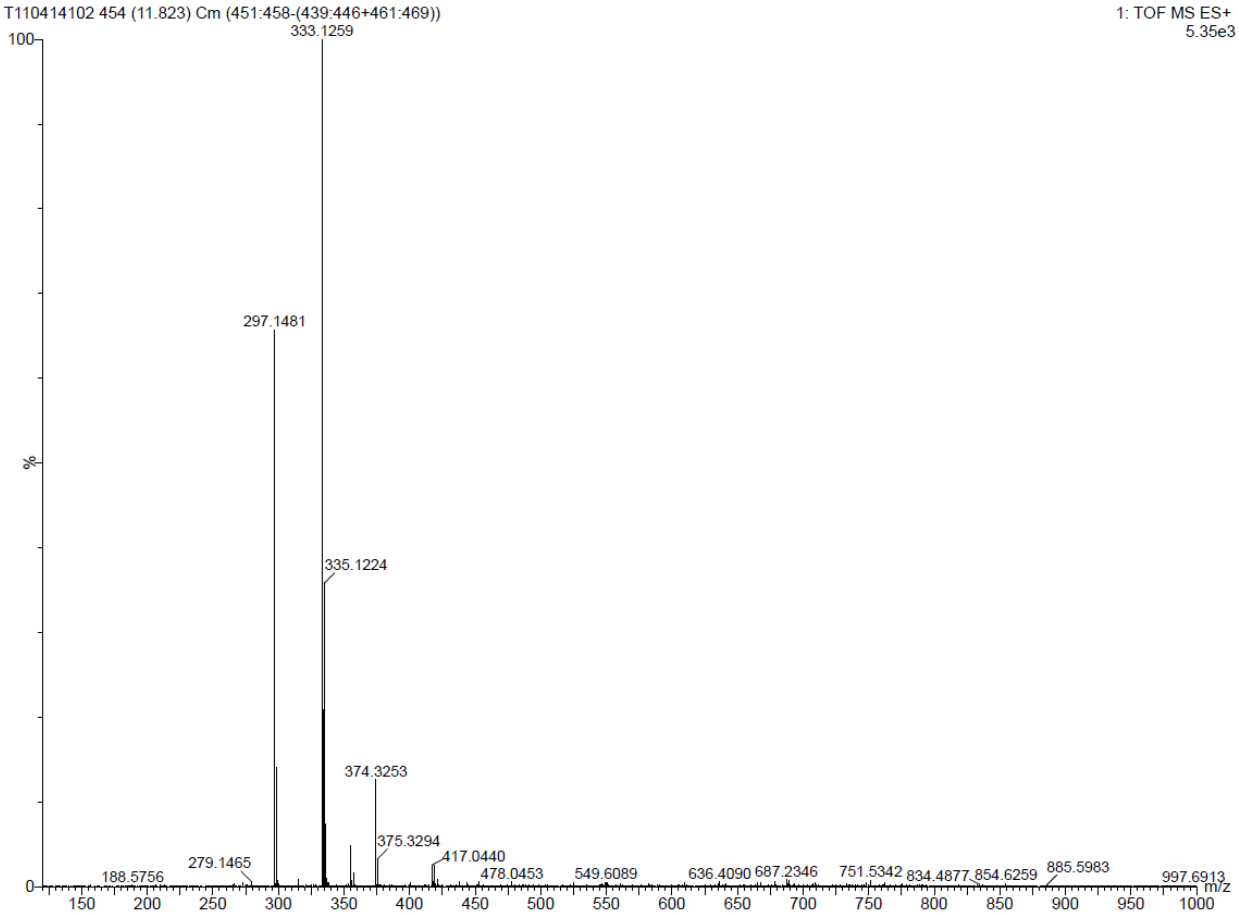


Figure S13: HRESITOFMS of chlorinated d1-adrenosterone product resulting from the reaction of prednisone with free chlorine.

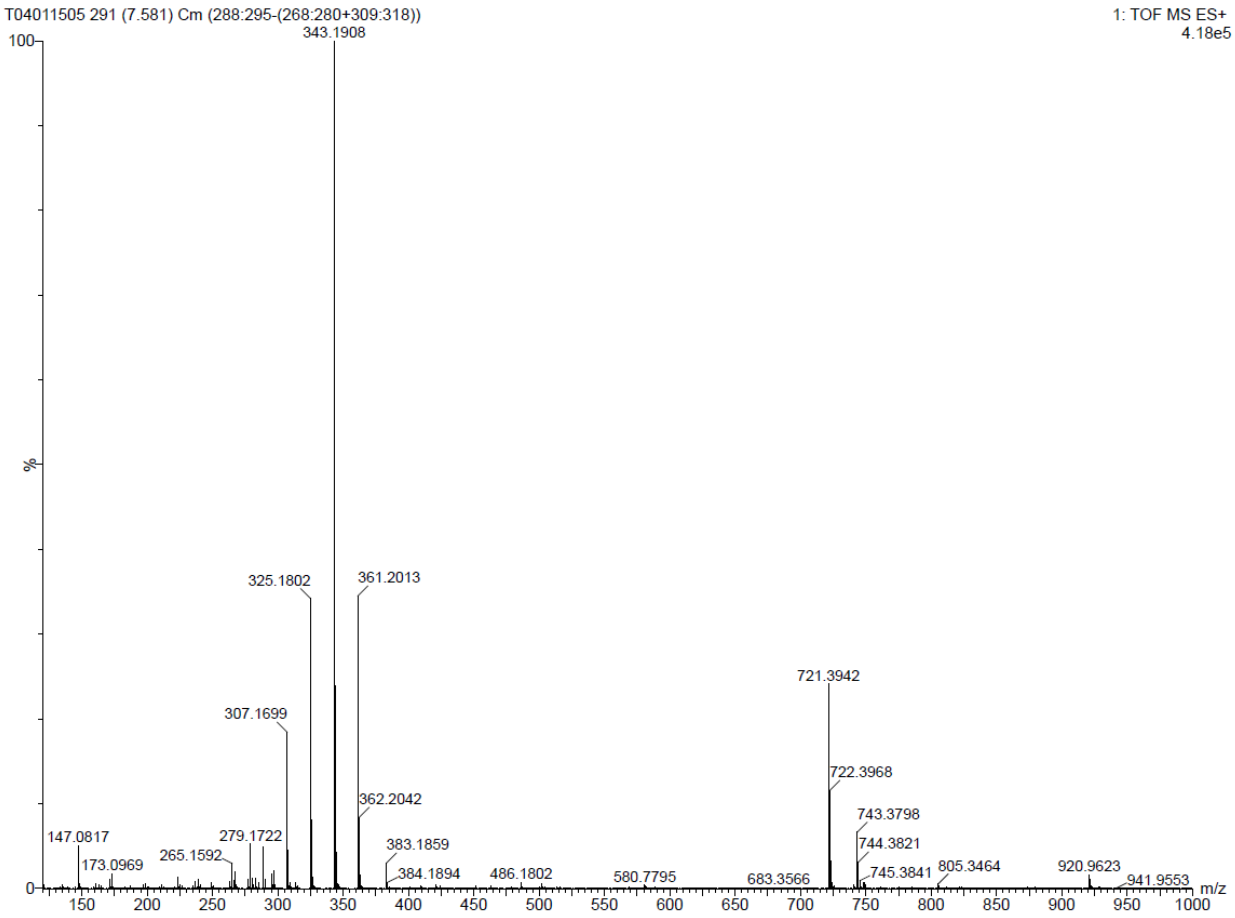


Figure S14: HRESITOFMS of prednisolone standard.

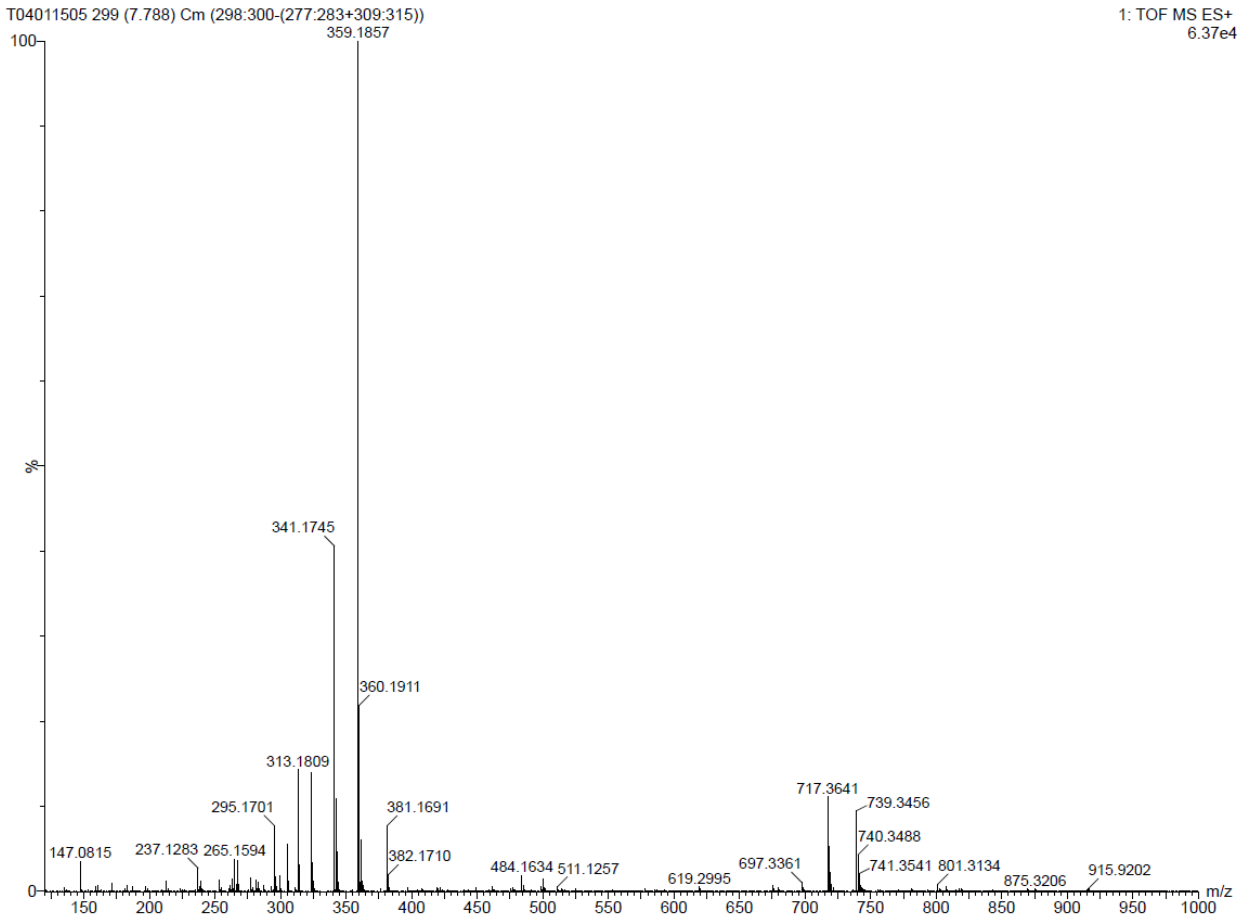


Figure S15: HRESITOFMS of prednisone product resulting from the reaction of prednisolone with free chlorine.

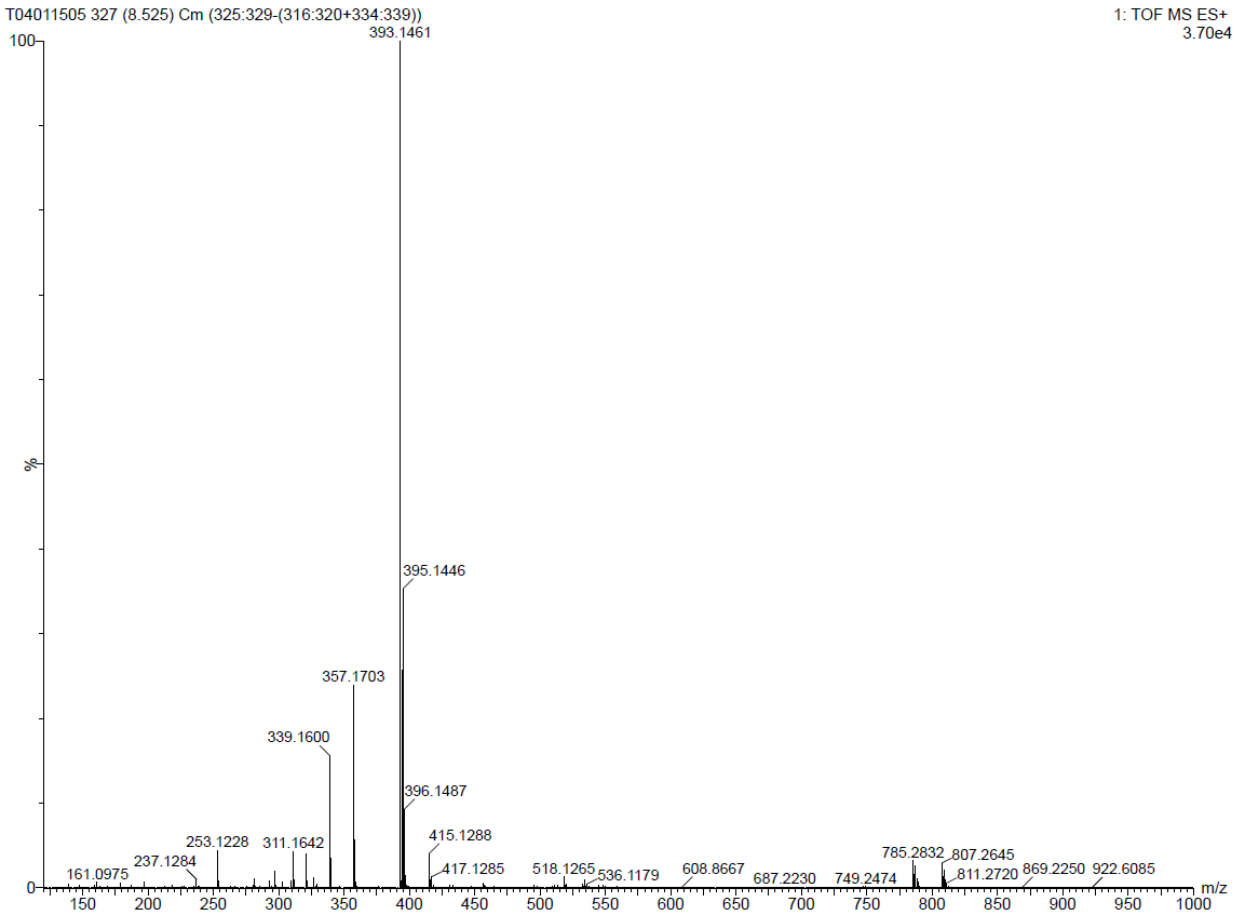


Figure S16: HRESITOFMS of chlorinated prednisone product resulting from the reaction of prednisolone with free chlorine.

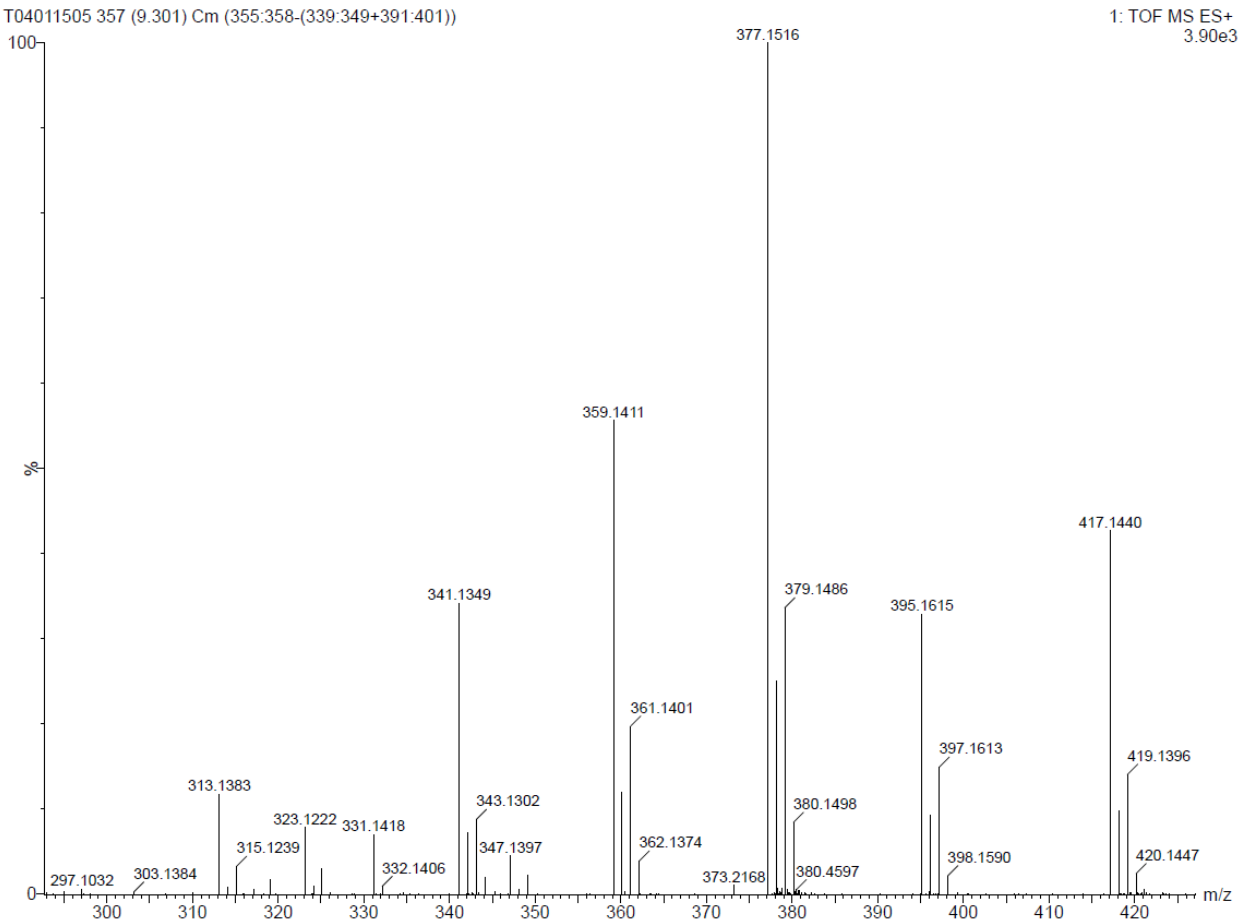


Figure S17: HRESITOFMS of chlorinated prednisolone product resulting from the reaction of prednisolone with free chlorine.

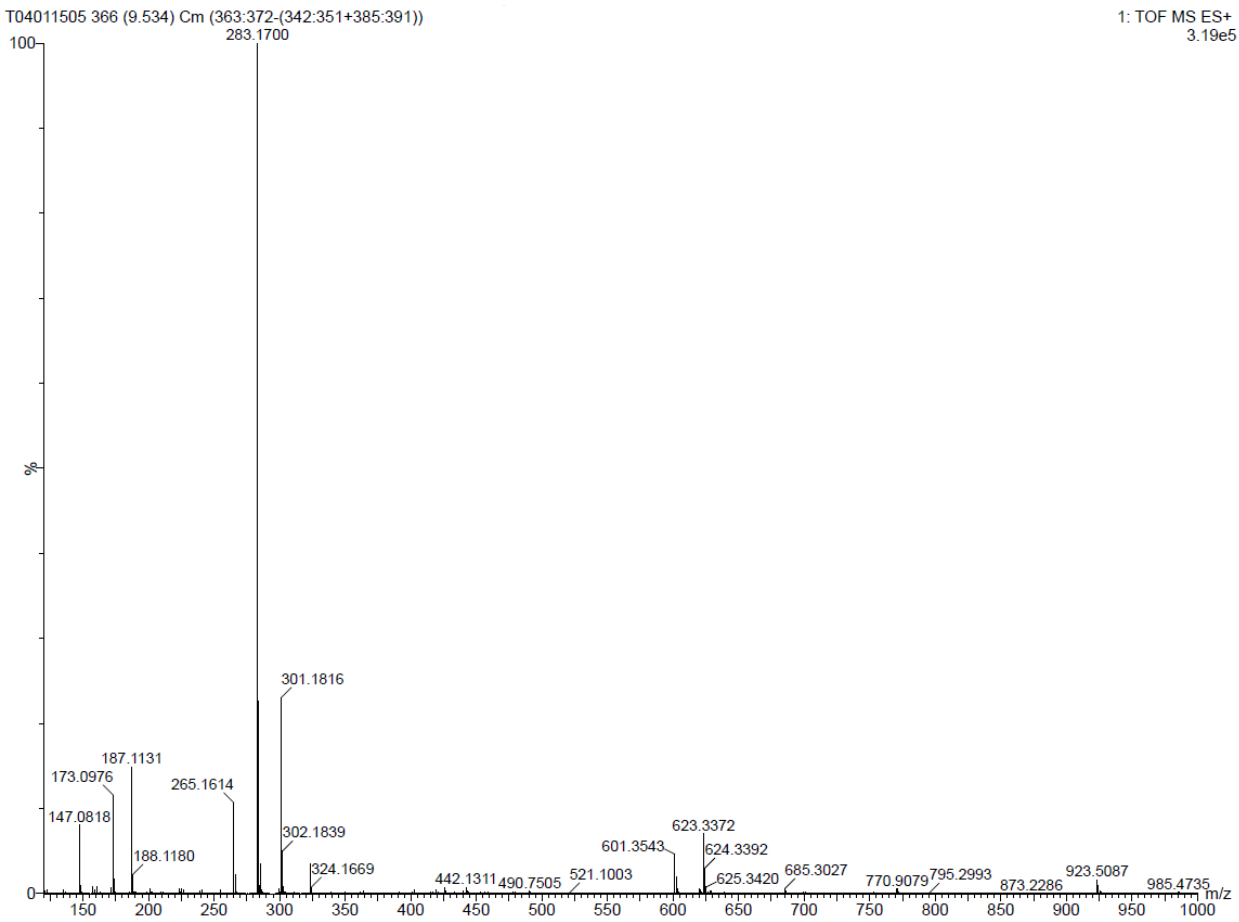


Figure S18: HRESITOFMS of 11β-hydroxyboldione product resulting from the reaction of prednisolone with free chlorine.

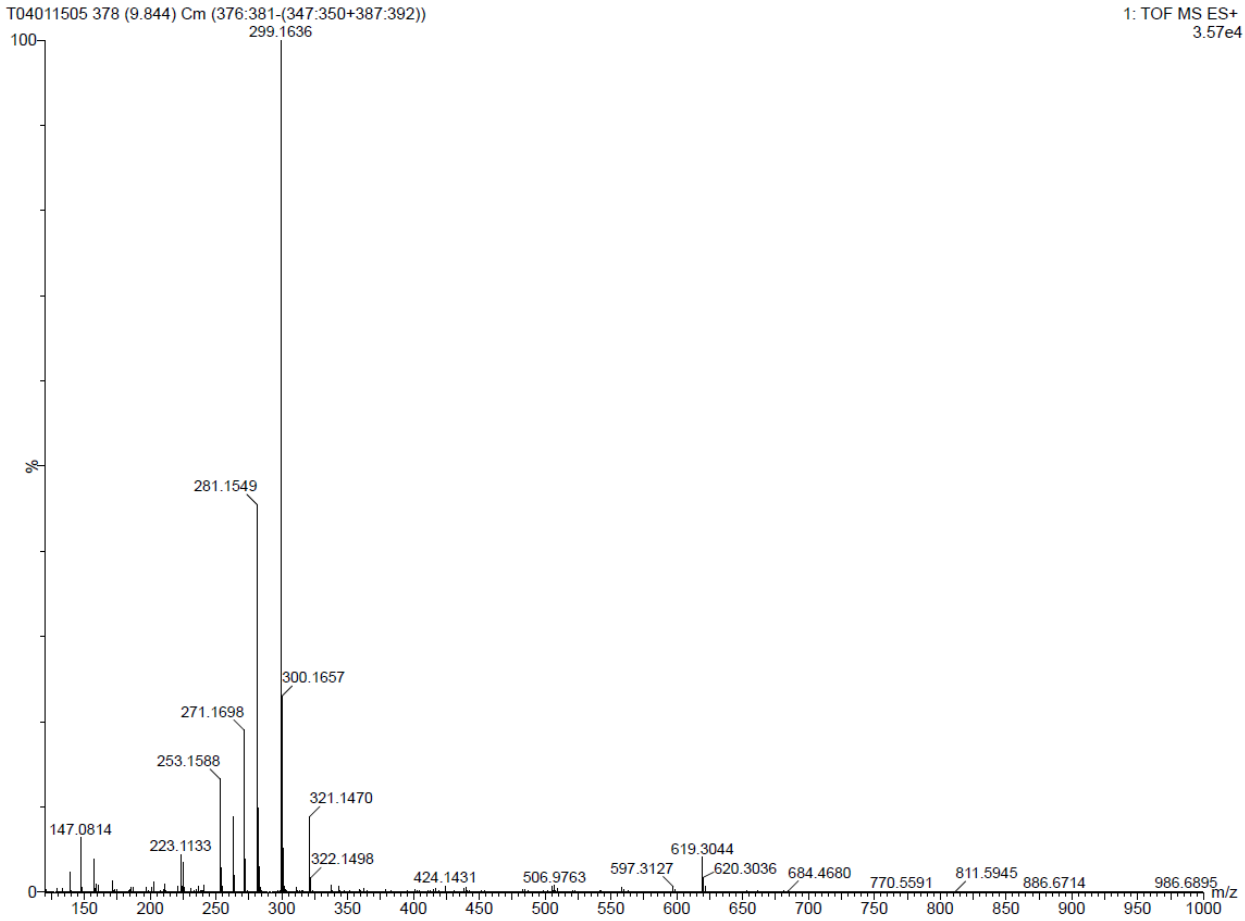


Figure S19: HRESITOFMS of d1-adrenosterone product resulting from the reaction of prednisolone with free chlorine.

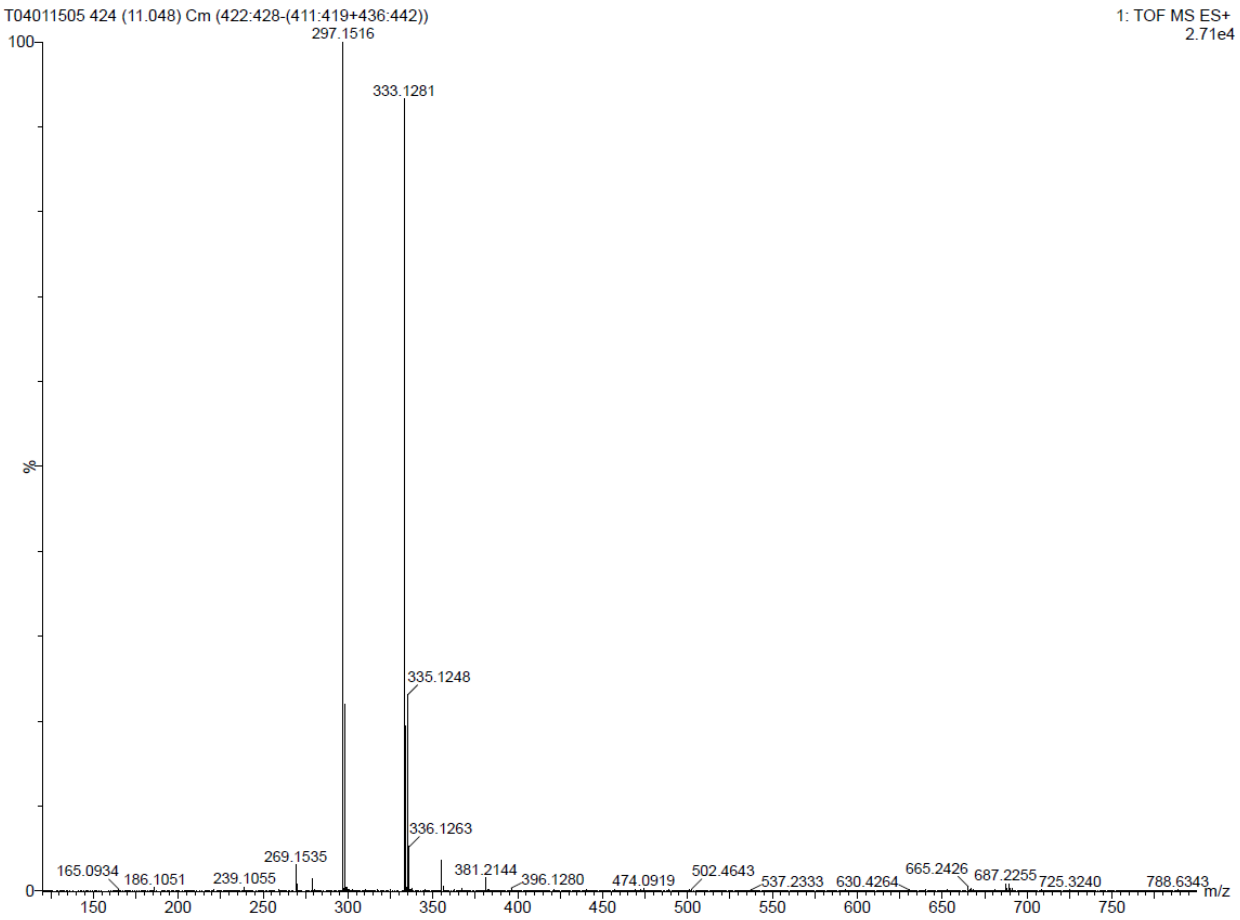


Figure S20: HRESITOFMS of chlorinated d1-adrenosterone product resulting from the reaction of prednisolone with free chlorine.

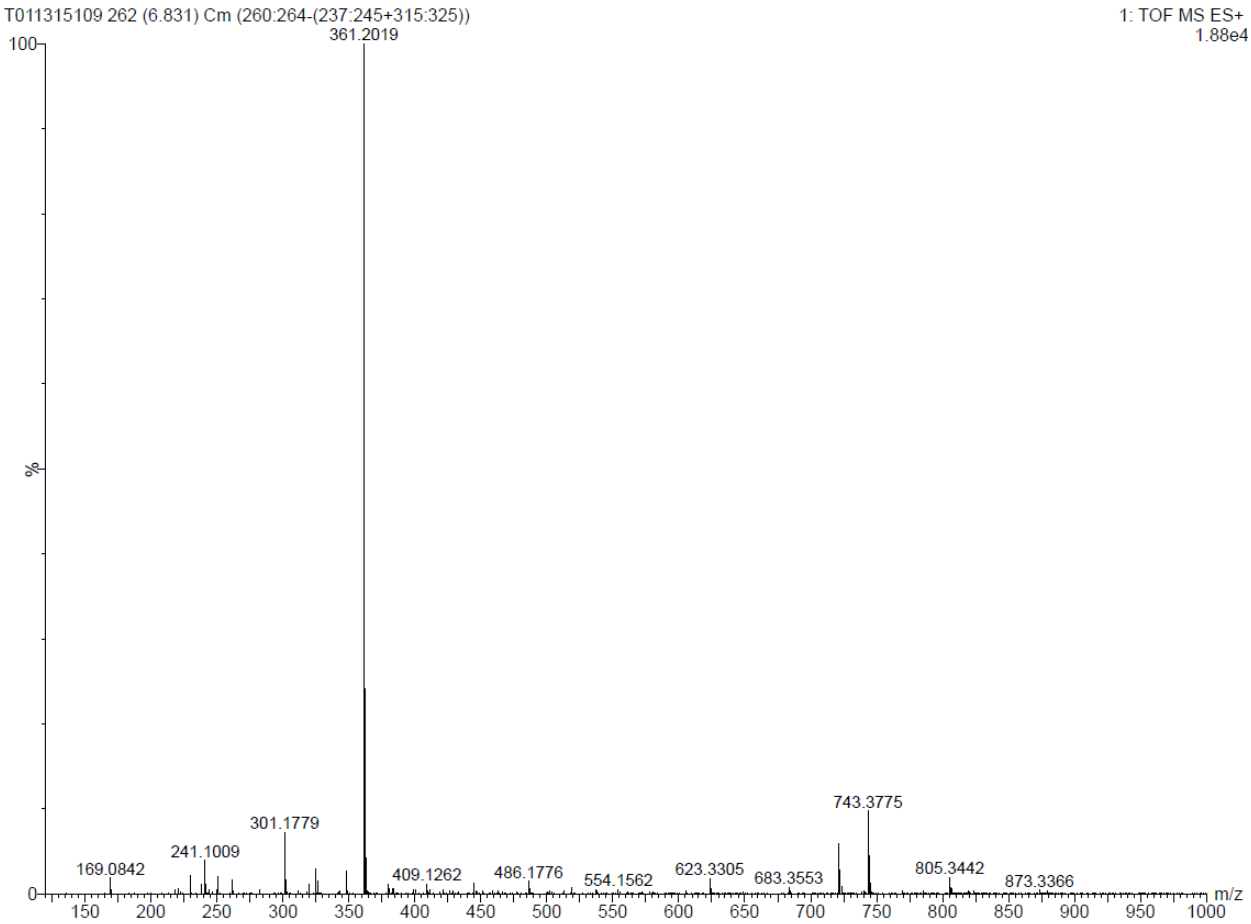


Figure S21: HRESITOFMS of cortisone standard.

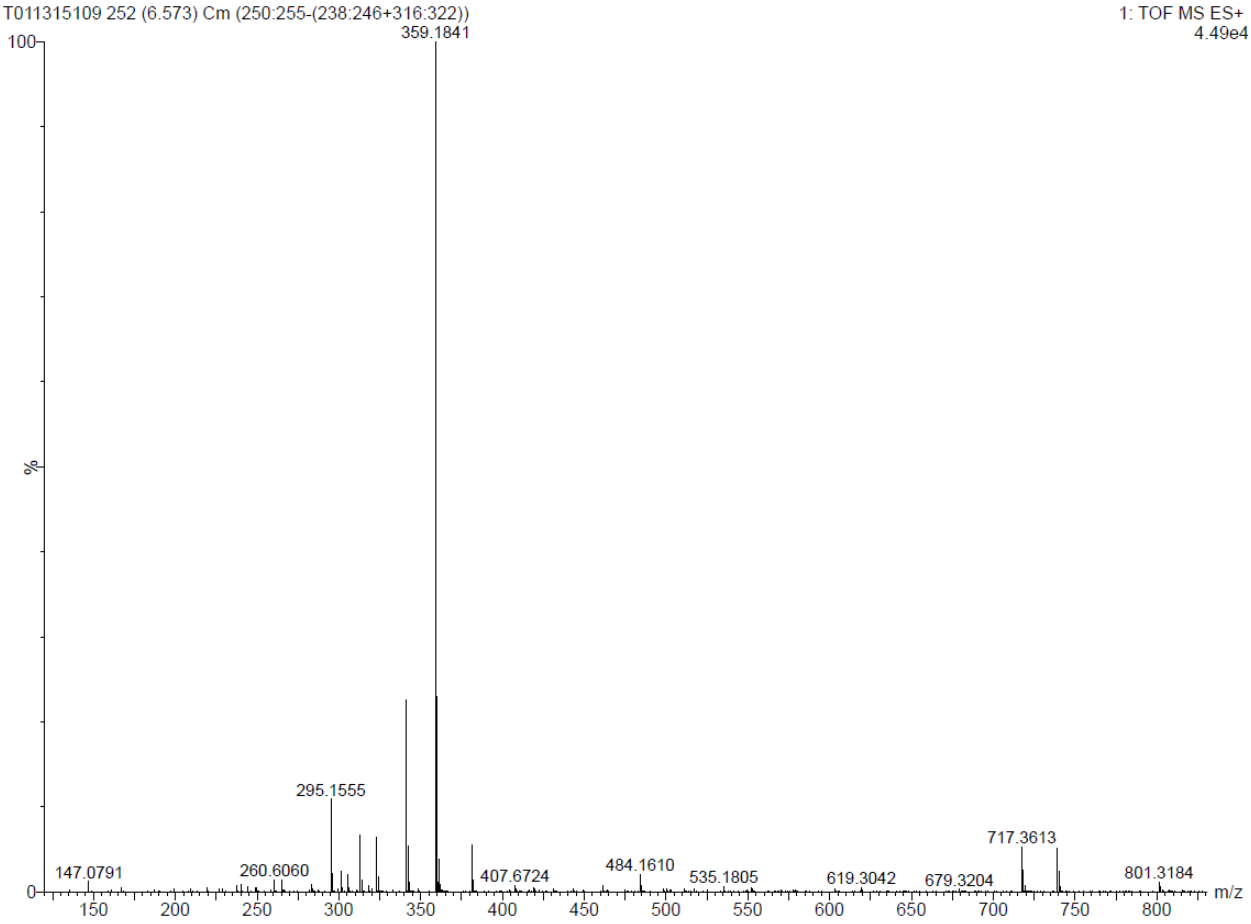


Figure S22: HRESITOFMS of prednisone product resulting from the reaction of cortisone with free chlorine.

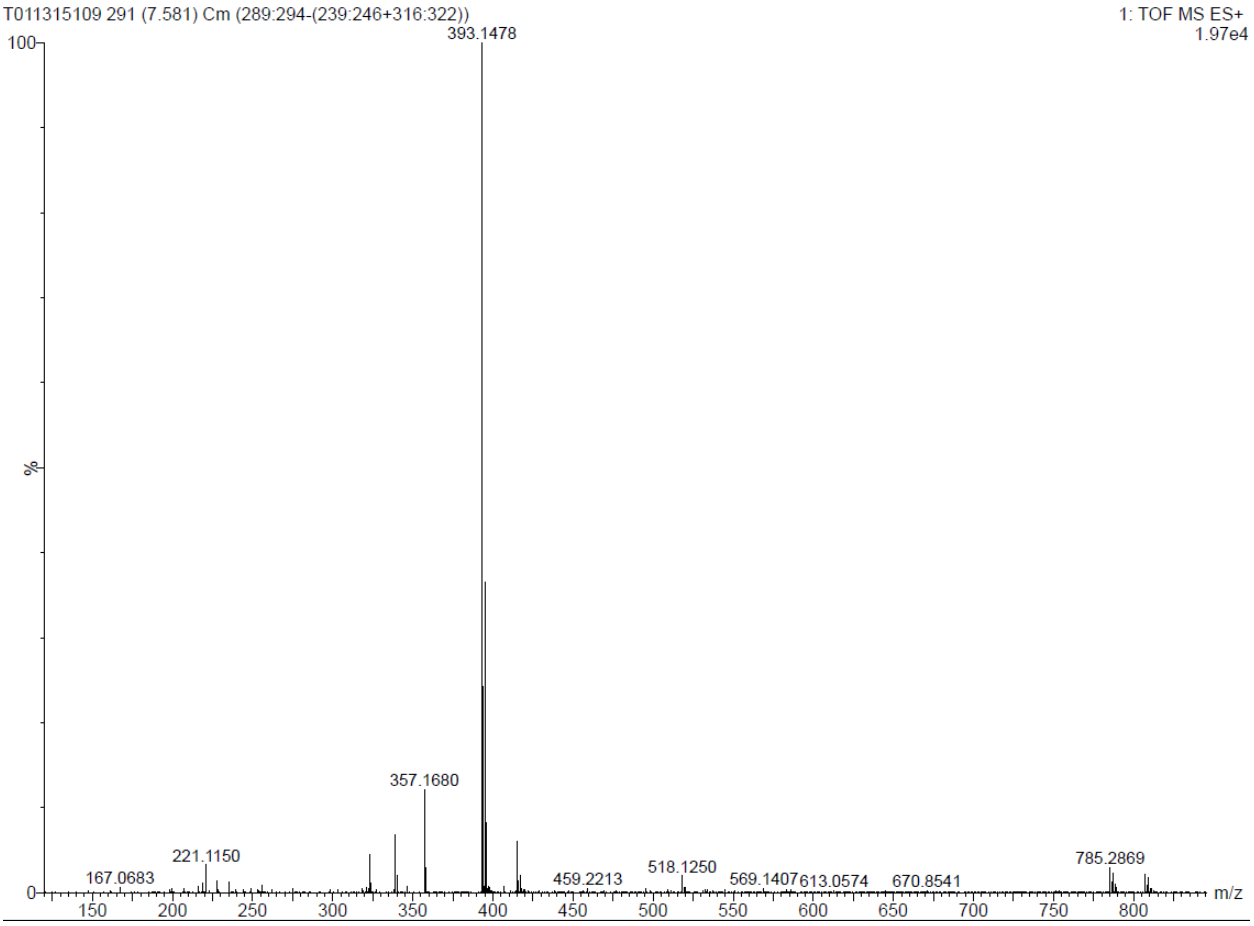


Figure S23: HRESITOFMS of chlorinated prednisone product resulting from the reaction of cortisone with free chlorine.

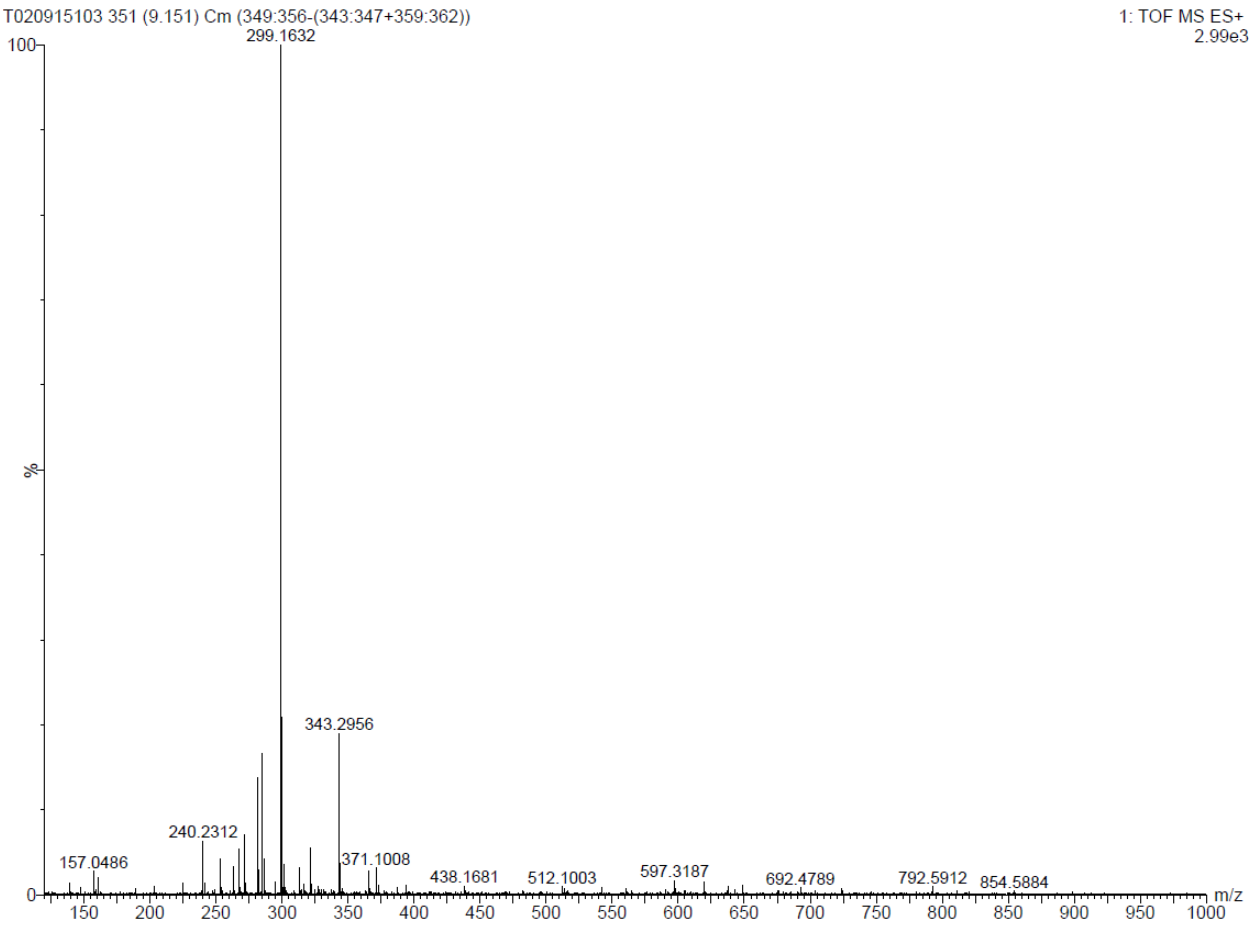


Figure S24: HRESITOFMS of d1-adrenosterone product resulting from the reaction of cortisone with free chlorine.

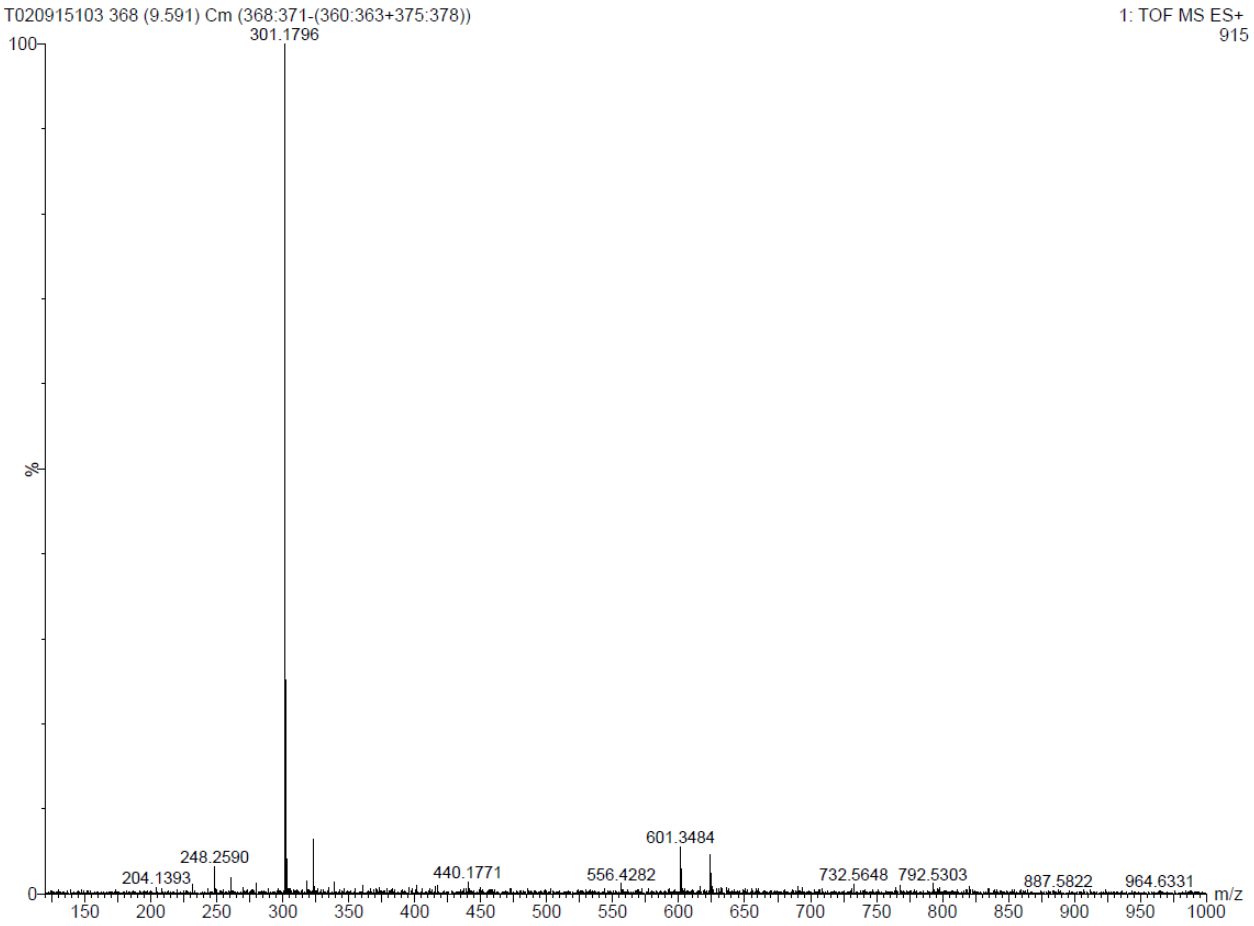


Figure S25: HRESITOFMS of adrenosterone product resulting from the reaction of cortisone with free chlorine.

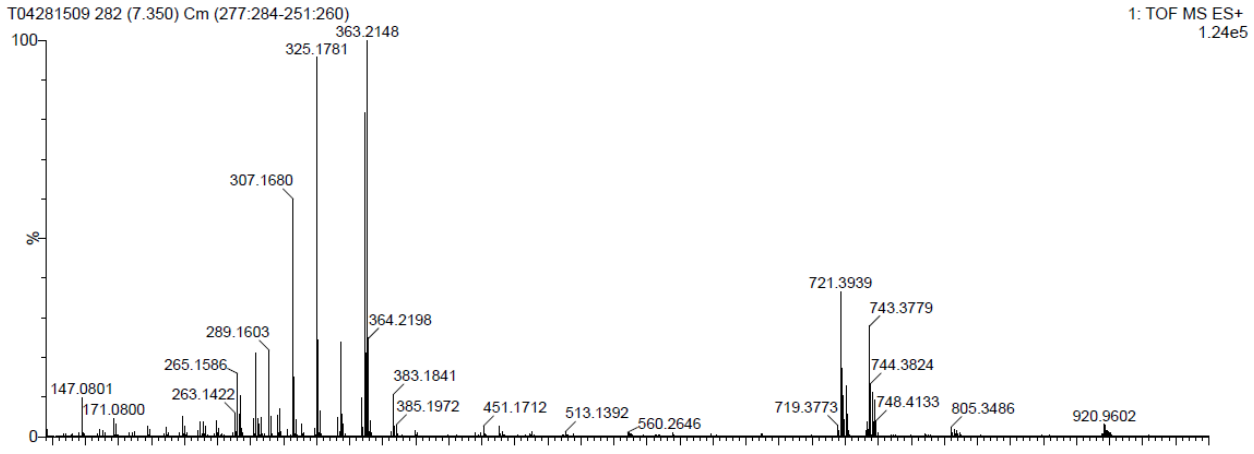


Figure S26: HRESITOFMS of cortisol standard.

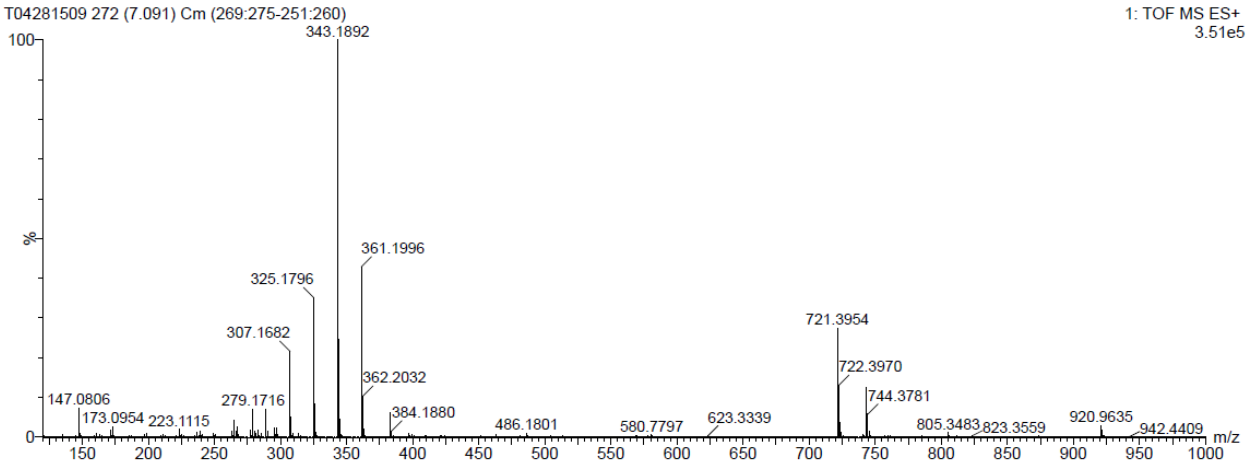


Figure S27: HRESITOFMS of prednisolone product resulting from the reaction of cortisol with free chlorine.

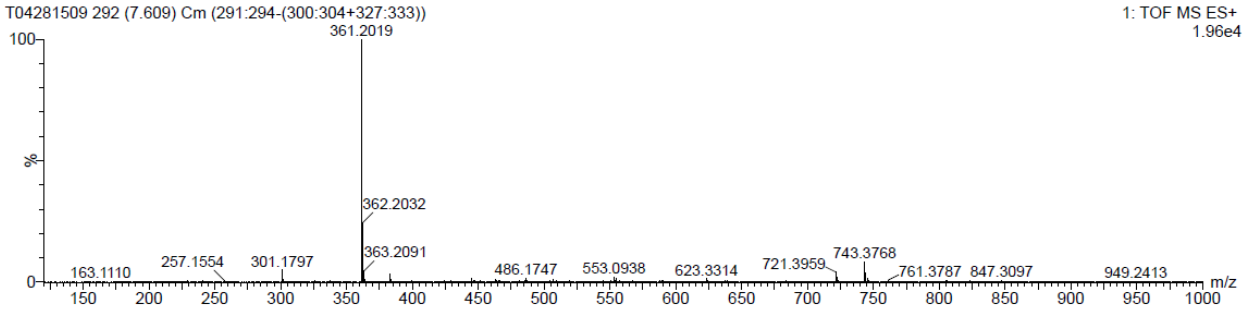


Figure S28: HRESITOFMS of cortisone product resulting from the reaction of cortisol with free chlorine.

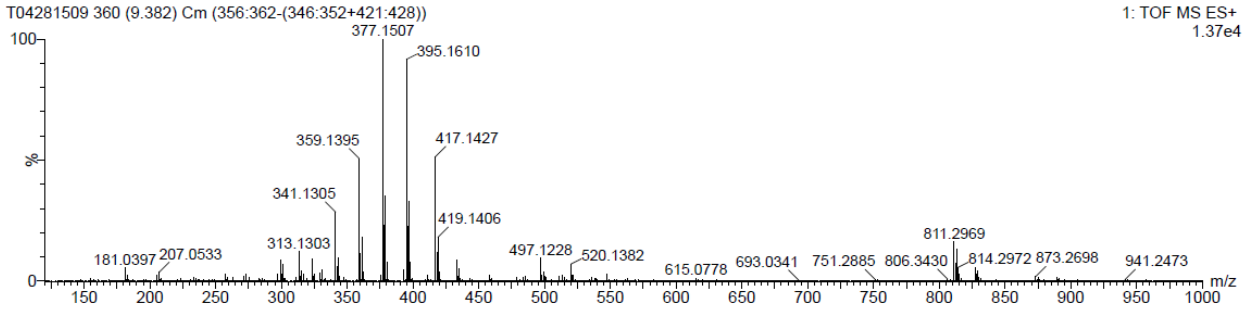


Figure S29: HRESITOFMS of chlorinated prednisolone product resulting from the reaction of cortisol with free chlorine.

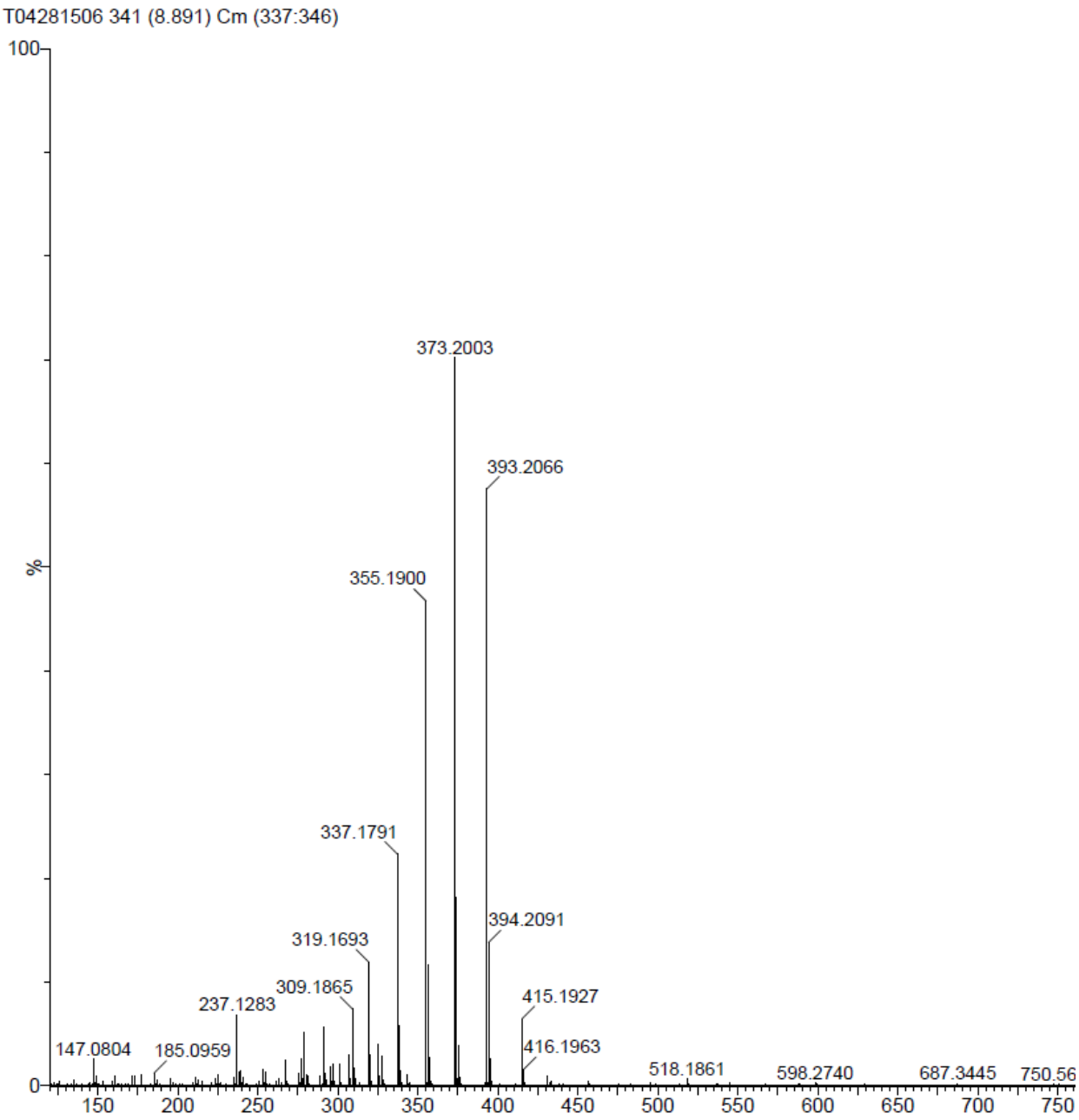


Figure S30: HRESITOFMS of dexamethasone standard.

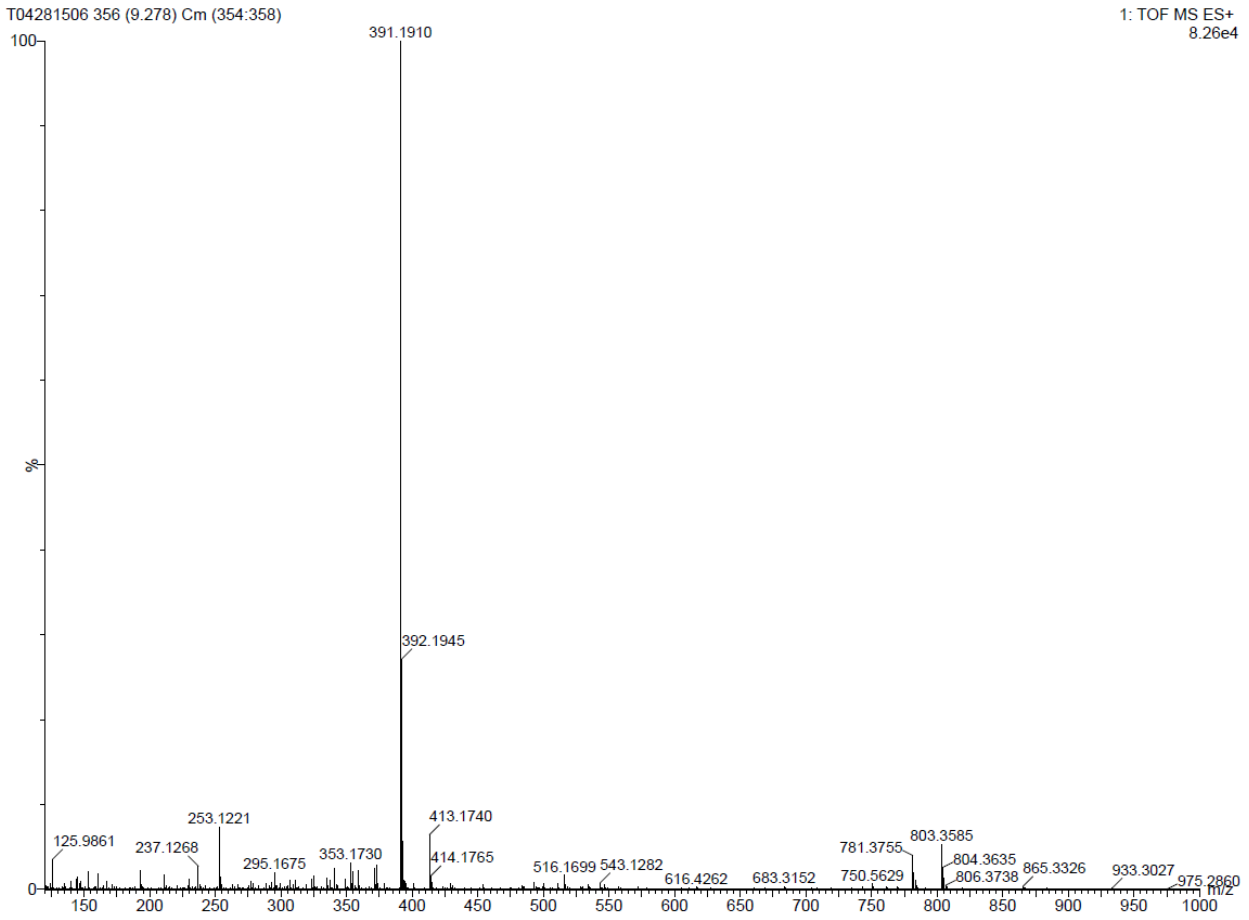


Figure S31: HRESITOFMS of 11-keto-dexamethasone product resulting from the reaction of dexamethasone with free chlorine.

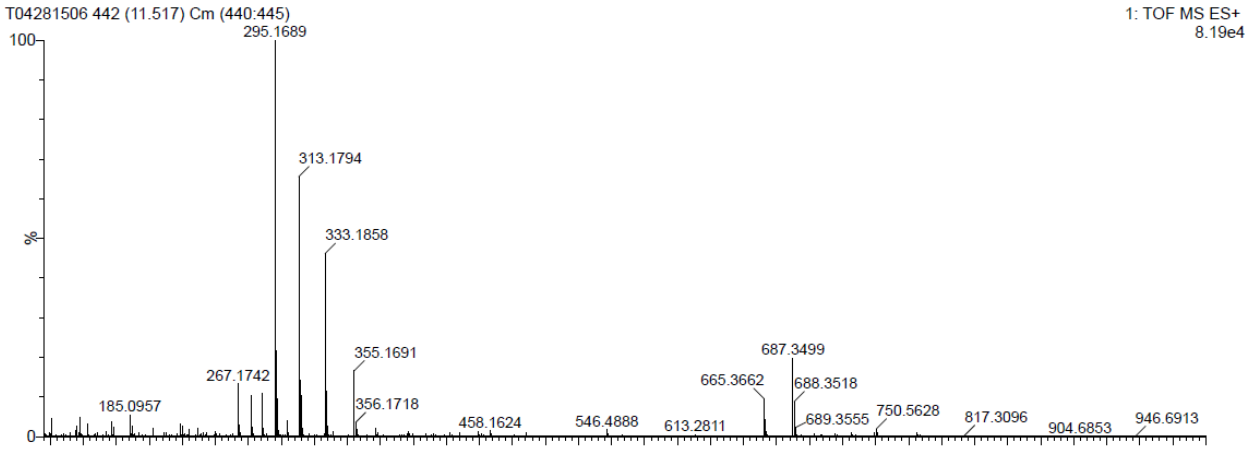


Figure S32: HRESITOFMS of 17-oxo-dexamethasone product resulting from the reaction of dexamethasone with free chlorine.