Electronic Supplementary Material (ESI) for Analyst.

Supporting Information

Simultaneous measurement of free and conjugated estrogens in surface water using capillary liquid chromatography tandem mass spectrometry

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List of supporting information:

Table S1. Physiochemical properties of free and conjugated estrogens.

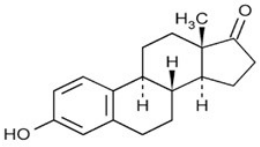
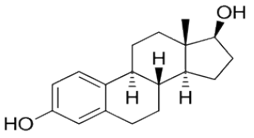
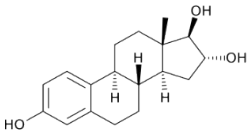
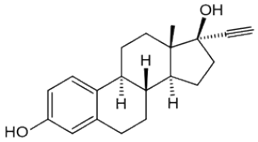
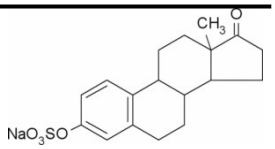
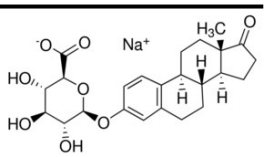
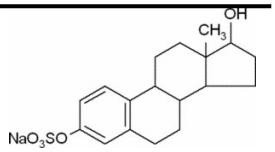
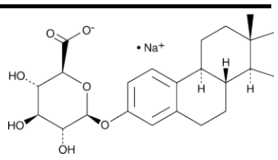
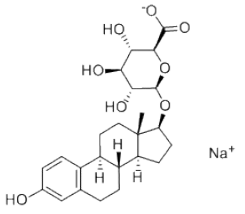
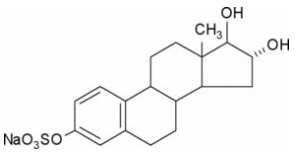
Estrogens	CAS	Molecular weight[a]	Solubility (mg mL^{-1}) [a/b]	Log P[a] (at 25°C)	pKa[c]	Chemical structure[a]
E1	53-16-7	270.37	Water: 1.3	3.13	10.25	
E2	50-28-2	272.38	Water: 1.51	4.01	10.27	
E3	50-27-1	288.4	Water: 13.25	2.45	10.25	
EE2	57-63-6	296.40	Water: 9.2	3.67	10.24	
E1-3S	438-67-5	372.41	Methanol: 19.6-20.4	2.5	-1.75	
E1-3G	15087-01-1	468.47	Water: 20	1.6	3.3	
E2-3S	4999-79-5	374.4	Methanol: 9.8-10.2	2.1	-1.8	
E2-3G	14982-12-8	470.49	Water: 10	1.1	3.3	
E2-17G	15087-02-2	470.49	0.1 M NaOH: 10	1.1	3.3	

Table S1. (continued)

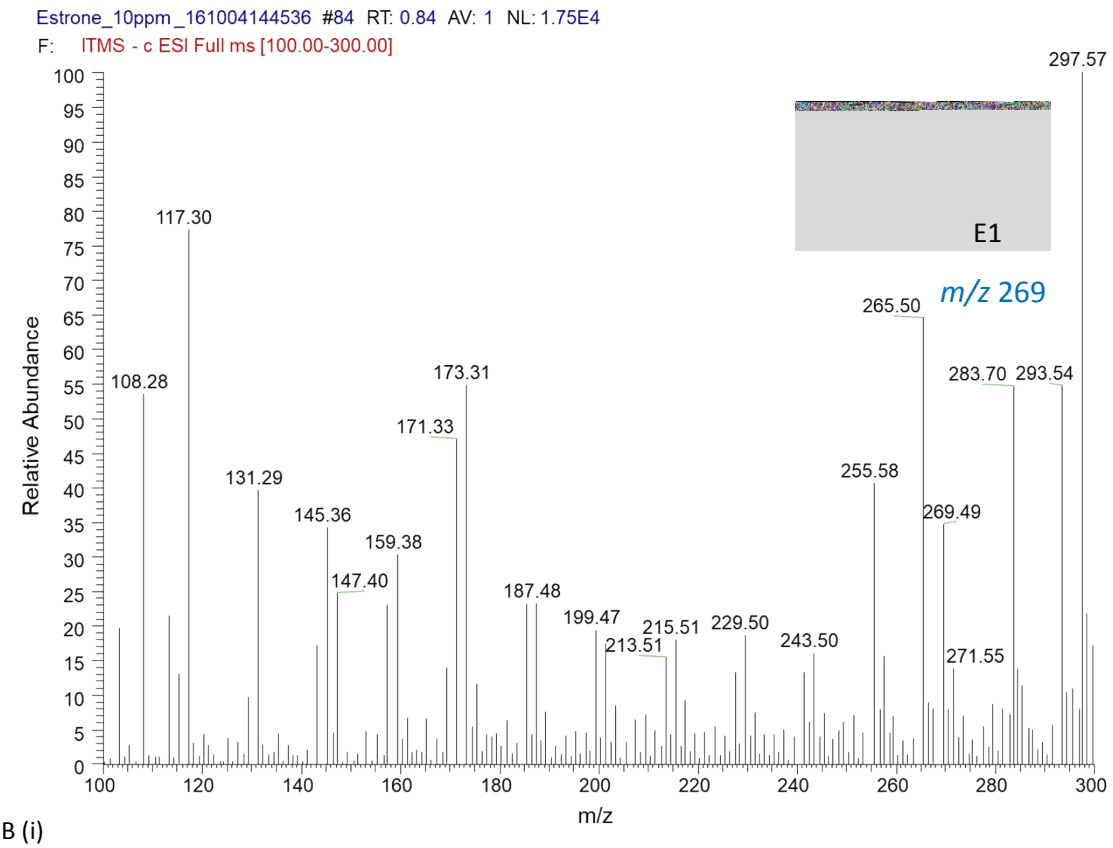
Estrogens	CAS	Molecular weight[a]	Solubility (mg mL^{-1}) [a]	Log P[a] (at 25°C)	pKa[b]	Chemical structure[a]
E3-3S	481-95-8	390.43	Methanol: 19.6-20.4	1.9	-1.7	

[a]Molecular weight (Mw), water solubility, partition coefficient (Log P) and structures are from PubChem and Sigma Adrich;

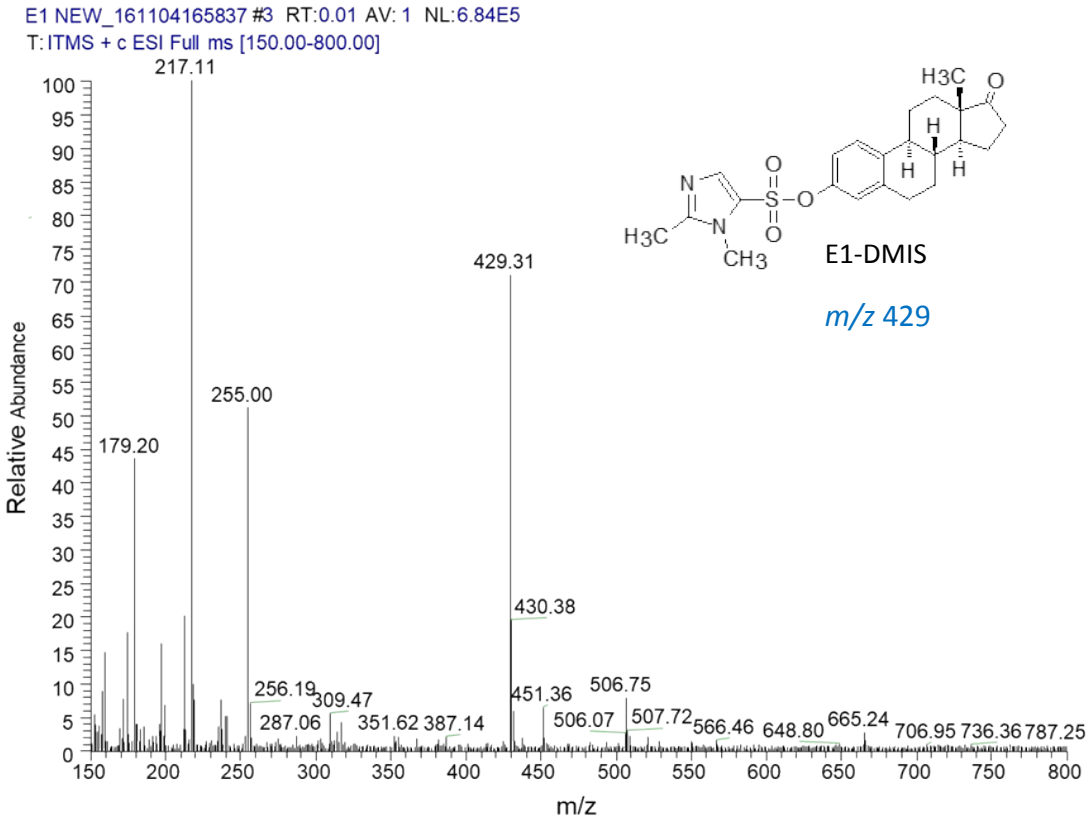
[b]water solubility of E1, E2 and EE2(Shareef, Angove, Wells, & Johnson, 2006)

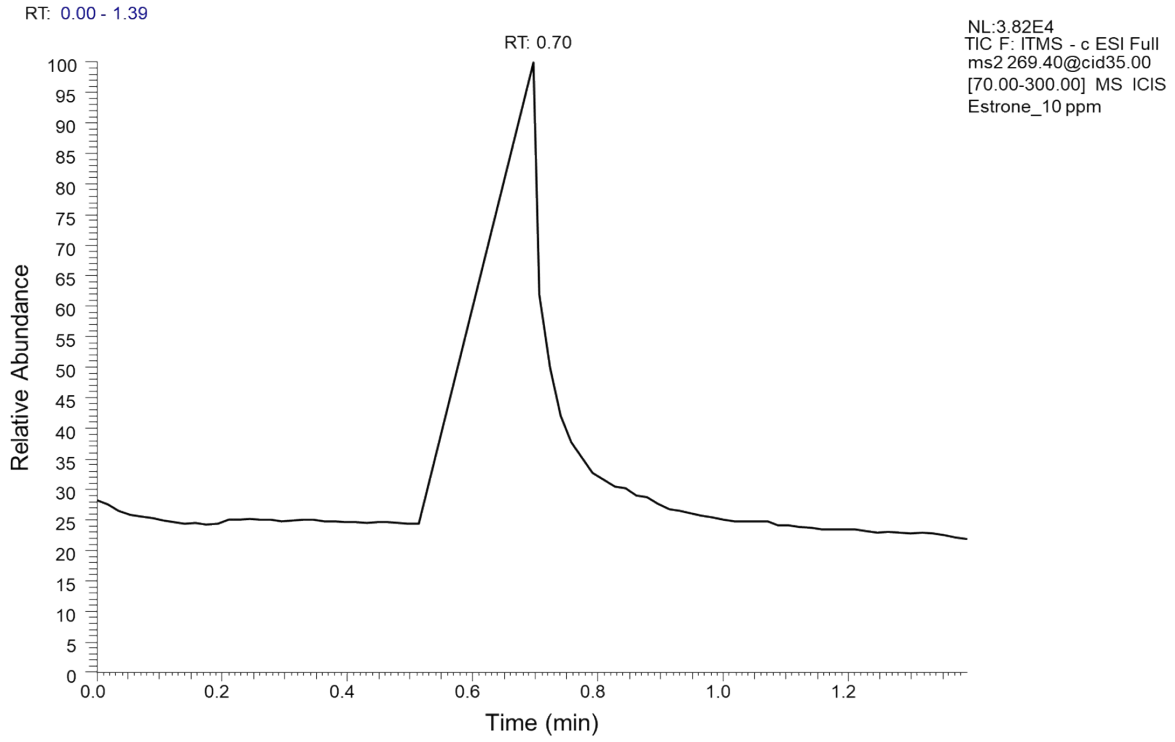
[c] dissociation constant (pKa) of estrogens (He & Aga, 2019; Tomšíková et al., 2012)

A (i)

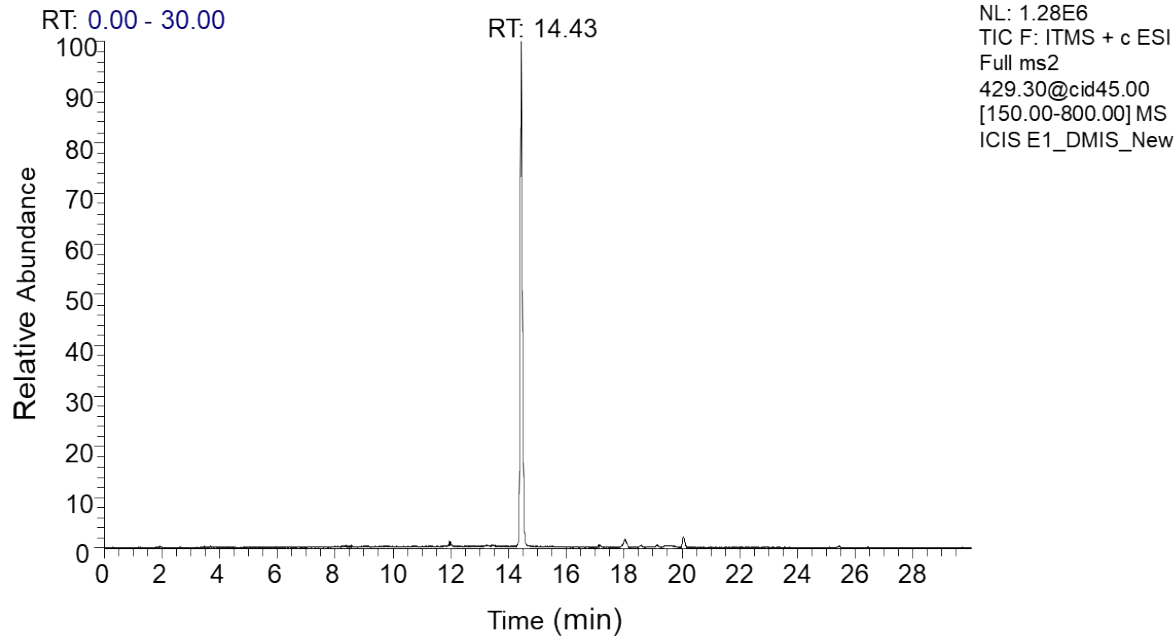

Estrone_10ppm_161004144536 #84 RT: 0.84 AV: 1 NL: 1.75E4
F: ITMS - c ESI Full ms [100.00-300.00]
Relative Abundance
m/z
297.57
117.30
108.28
173.31
171.33
131.29
145.36
159.38
147.40
187.48
199.47
213.51
215.51
229.50
243.50
265.50
255.58
269.49
271.55
283.70
293.54
E1
m/z 269

B (i)


E1 NEW_161104165837 #3 RT:0.01 AV: 1 NL:6.84E5
T: ITMS + c ESI Full ms [150.00-800.00]
Relative Abundance
m/z
217.11
429.31
255.00
179.20
430.38
256.19
309.47
287.06
351.62
387.14
451.36
506.75
506.07
507.72
566.46
648.80
665.24
706.95
736.36
787.25
E1-DMIS
m/z 429



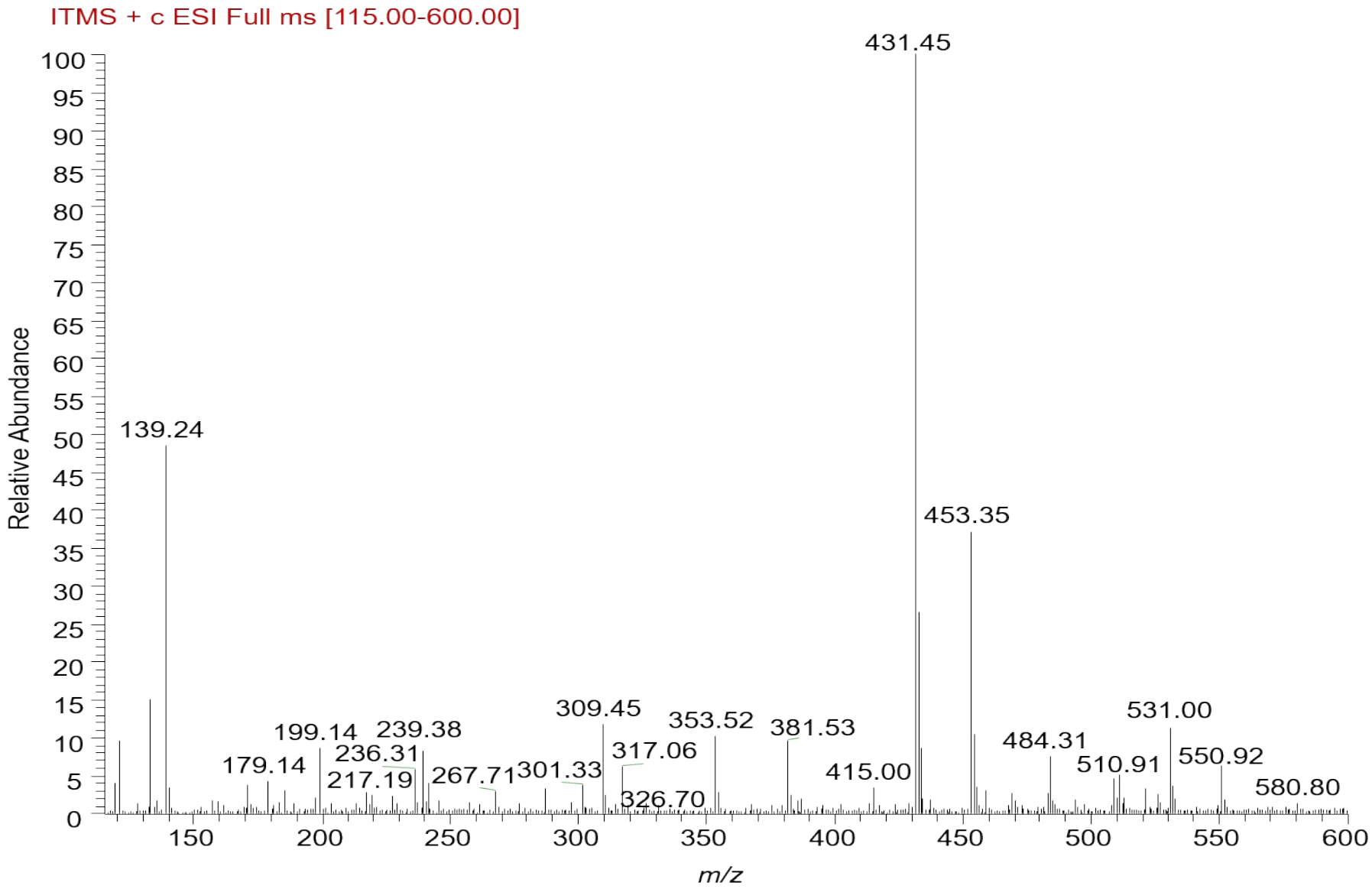
A (ii)



B (ii)

Figure S1. ESI mass spectra acquired using a direct injection through a syringe A(i) E1 at 10 mg L^{-1} without derivatisation reaction; B(i) E1 at 0.1 mg L^{-1} after derivatisation reaction with DMIS;

Reconstructed ion chromatogram (RIC) for precursor ions corresponding to A(ii) free estrogen E1 without DMIS derivatisation and B(ii)— with derivatisation of E1-DMIS


ITMS + c ESI Full ms [115.00-600.00]
Relative Abundance
m/z
431.45
139.24
453.35
309.45
353.52
381.53
531.00
484.31
199.14
239.38
236.31
317.06
550.92
510.91
179.14
217.19
267.71
301.33
415.00
326.70
580.80

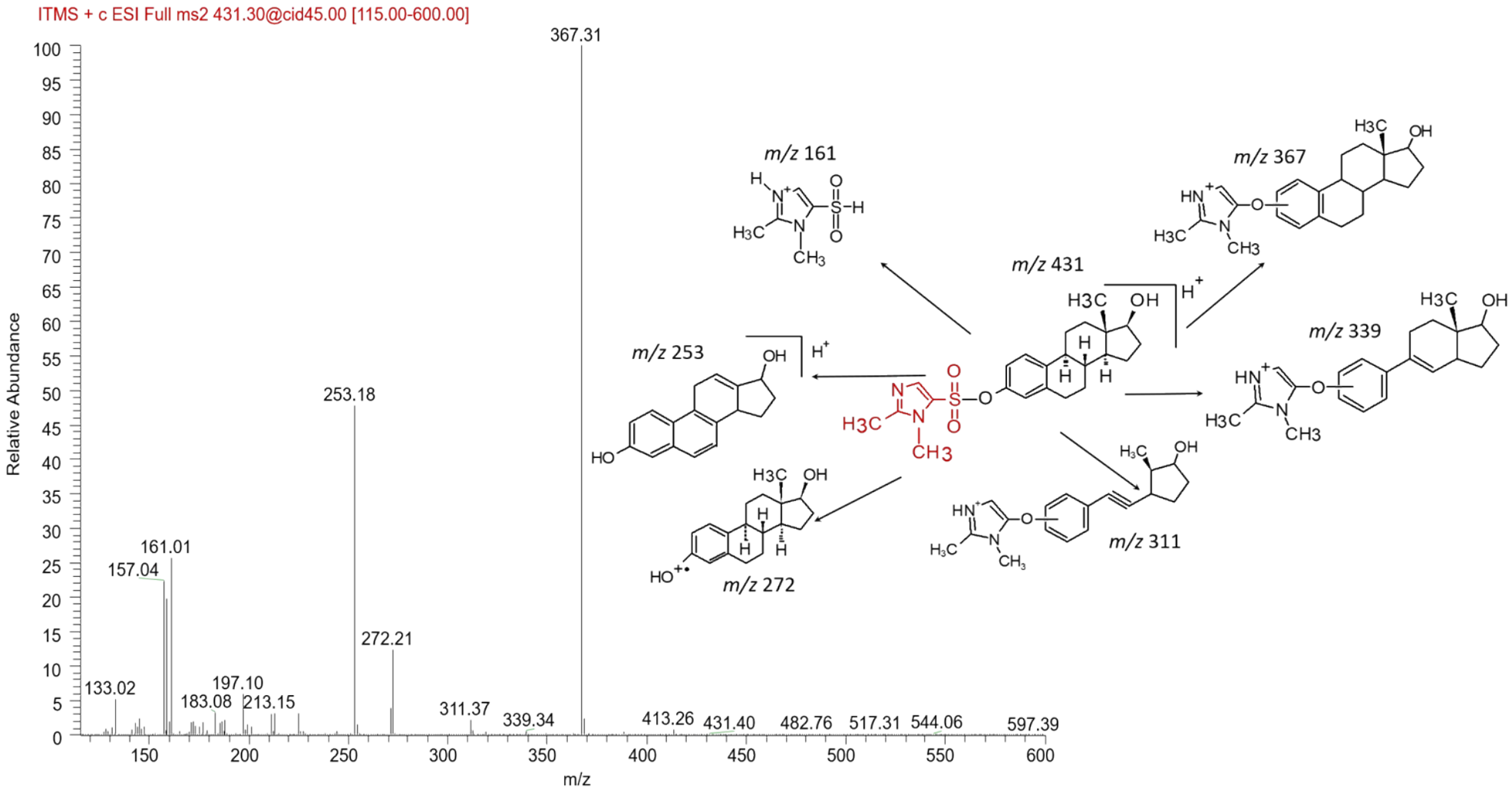
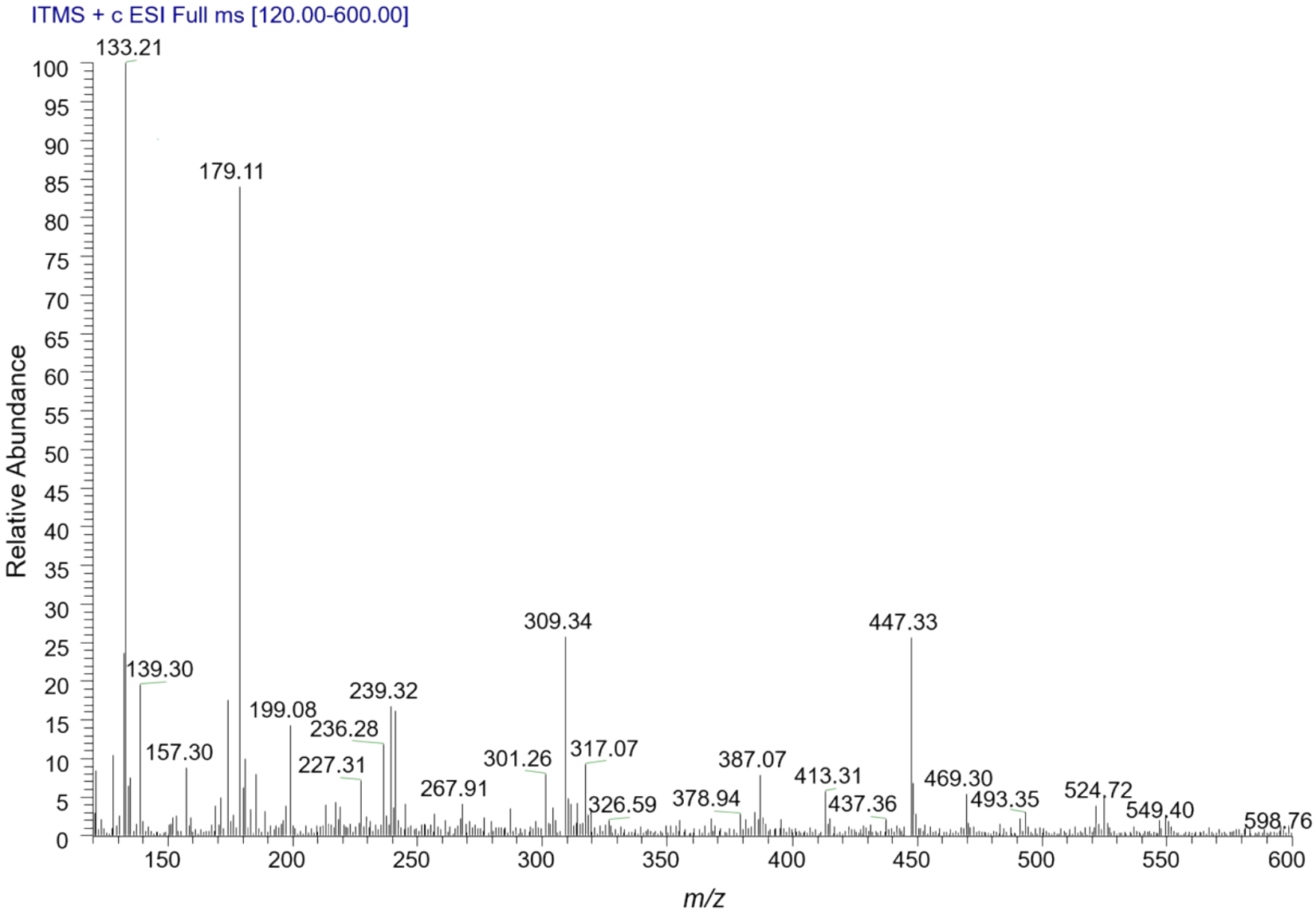


Figure S2. (a) Full MS and MS^2 (431→) spectra of E2-DMIS and its fragmentation pathway.


ITMS + c ESI Full ms [120.00-600.00]
Relative Abundance
m/z
133.21
179.11
139.30
157.30
199.08
236.28
239.32
227.31
267.91
301.26
309.34
317.07
326.59
378.94
387.07
413.31
437.36
447.33
469.30
493.35
524.72
549.40
598.76

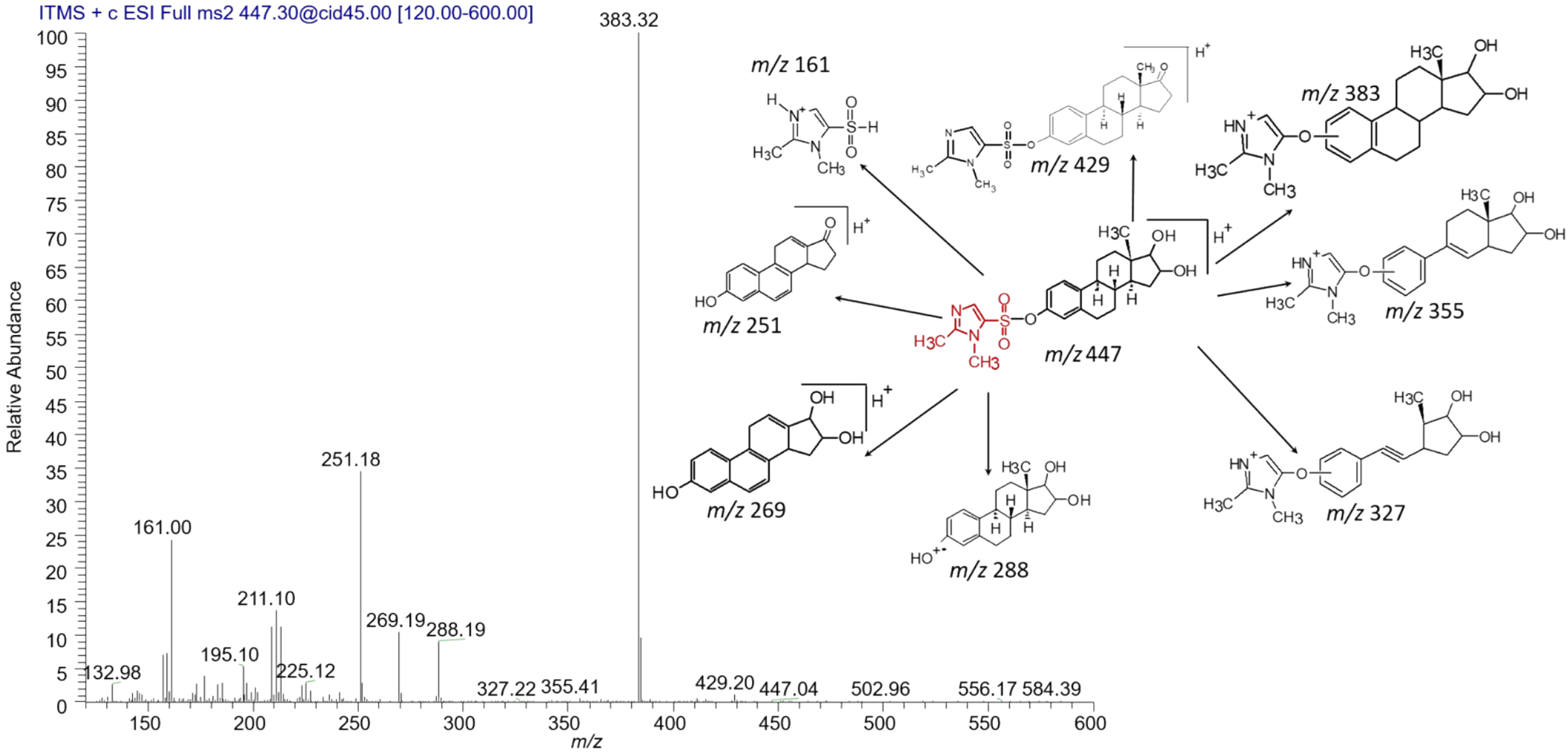
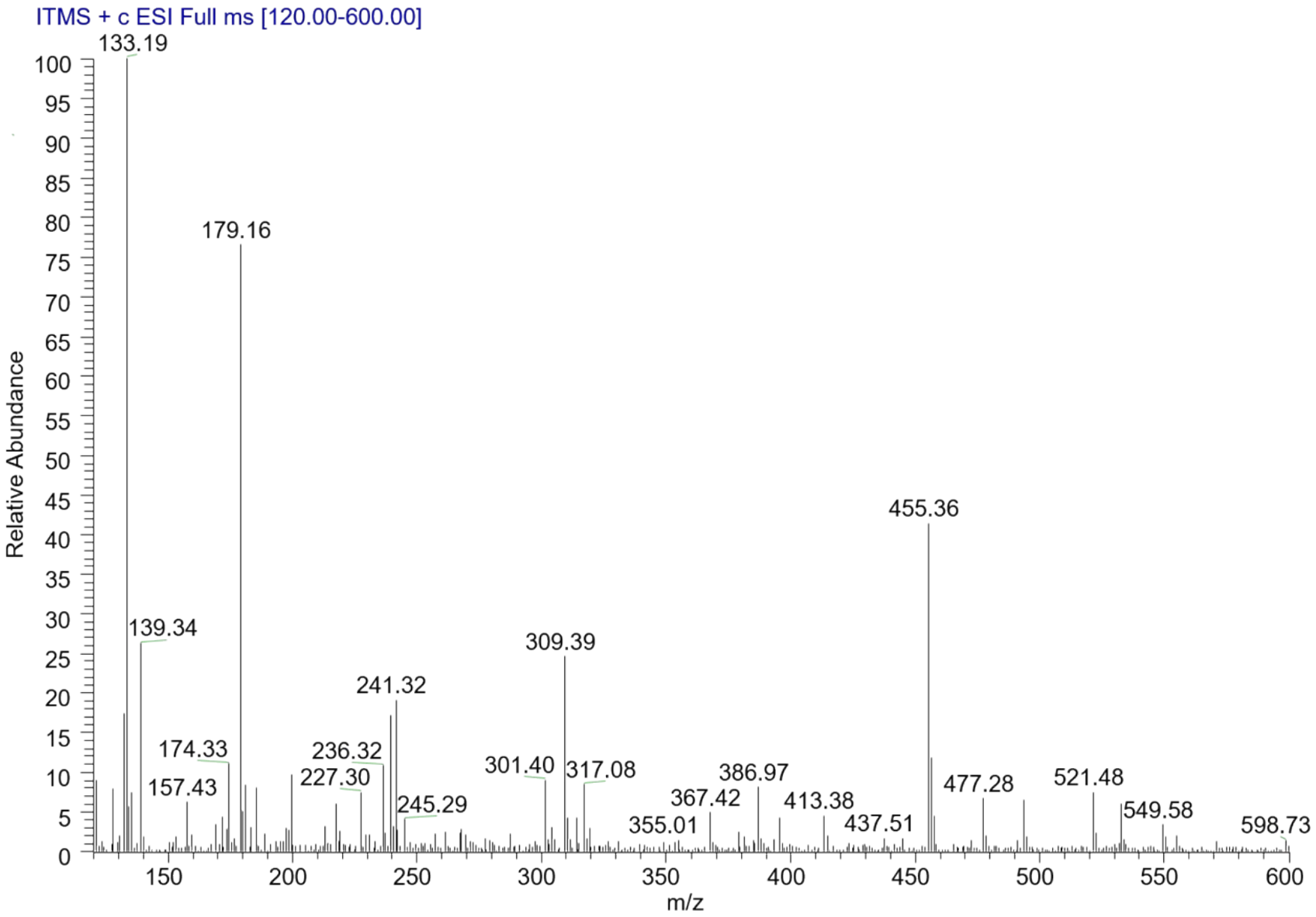


Figure S2. (b) Full MS and MS^2 (447→) of E3-DMIS and its fragmentation pathway.


ITMS + c ESI Full ms [120.00-600.00]
Relative Abundance
m/z
133.19
179.16
139.34
309.39
241.32
174.33
236.32
227.30
301.40
317.08
386.97
157.43
367.42
413.38
245.29
355.01
437.51
455.36
477.28
521.48
549.58
598.73

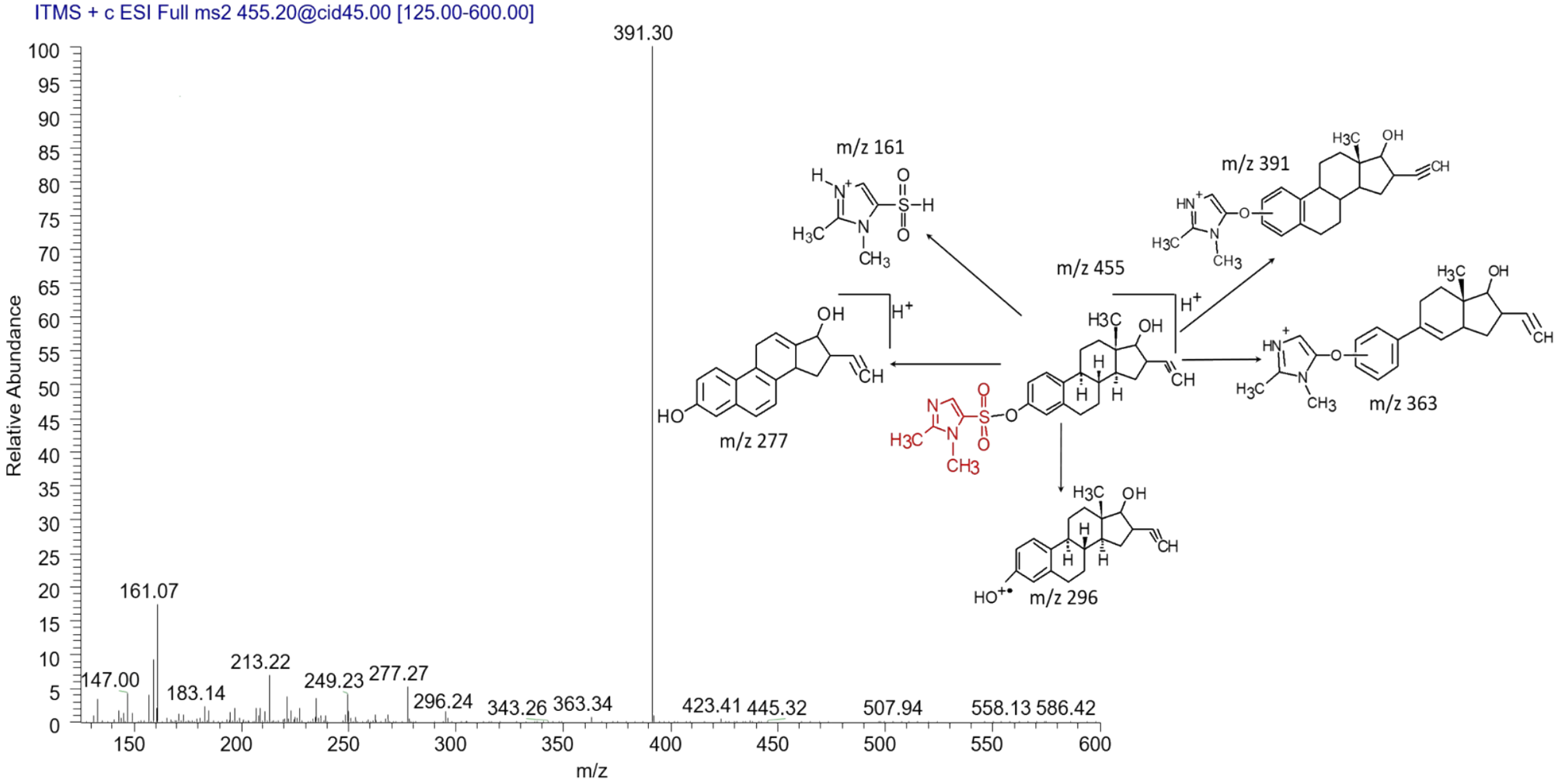
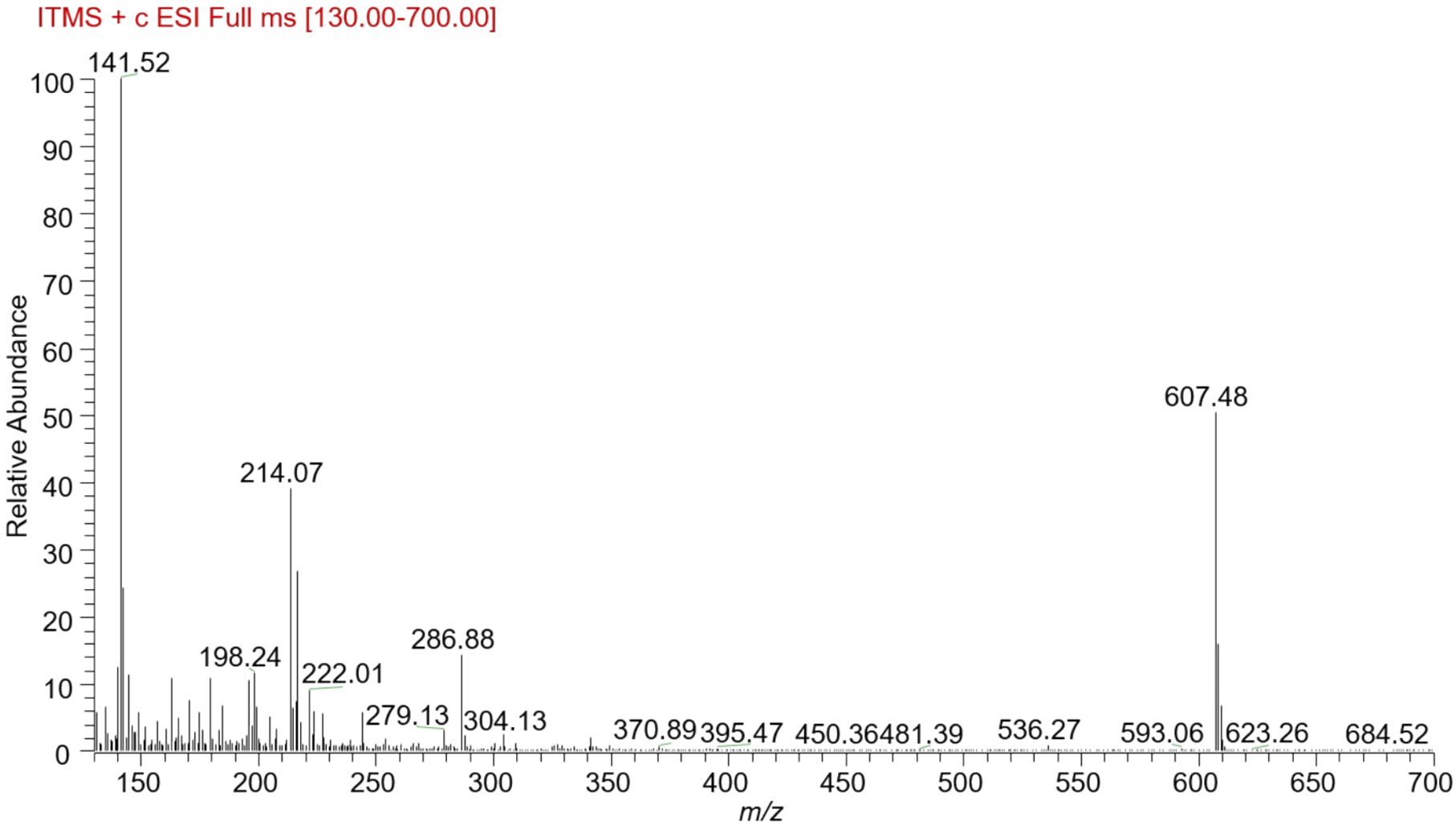


Figure S2. (c) Full MS and MS^2 (455→) of EE2-DMIS and its fragmentation pathway.


ITMS + c ESI Full ms [130.00-700.00]
Relative Abundance
m/z
141.52
214.07
607.48
198.24
222.01
286.88
279.13
304.13
370.89
395.47
450.36
481.39
536.27
593.06
623.26
684.52

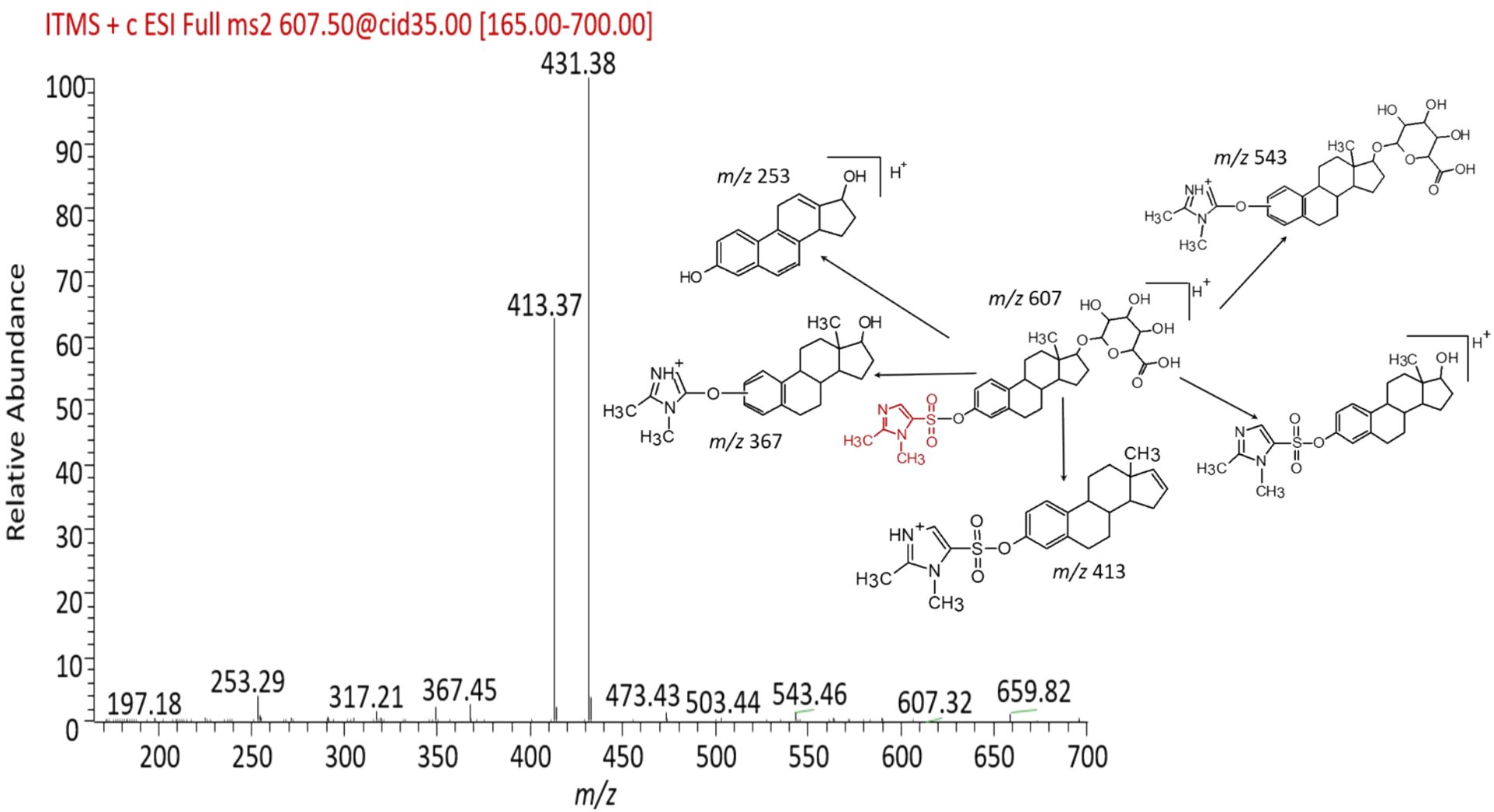


Figure S2. (d) Full MS and MS^2 (607→) spectra of E2-17G-DMIS and its fragmentation pathway.

Text S1. Preparation of DMIS solution.

DMIS solution at 1 mg mL^{-1} was prepared via taking 10 mg DMIS solid which was usually airtight sealed and stored at 5°C, and dissolving it in 10 mL acetone. Then the mixture was homogenised using vortex mixer. Prior to carrying out the derivatization procedure for a batch of samples, fresh DMIS solution was prepared.

Text S1. Optimisation of derivatisation method.

Conditions such as derivatisation buffers (ammonium formate and sodium bicarbonate) with the same pH value around 10.5, the amount of 1,2-dimethyl-1H-imidazole-5-sulphonyl chloride (DMIS) used for derivatising estrogens (30, 50, 75, 100 µL), temperature applied for yielding the most derivatives within 15 min (15, 30, 45, 60 and 75℃) and reaction time (10, 15, 30, 45 and 60 min) were tested. Finally, the optimum procedure was established by mixing 75 µL DMIS and 75 µL 50 mM sodium bicarbonate buffer (pH =10.5) with estrogens together and heating mixed solution at 60℃ for 15 min afterwards.

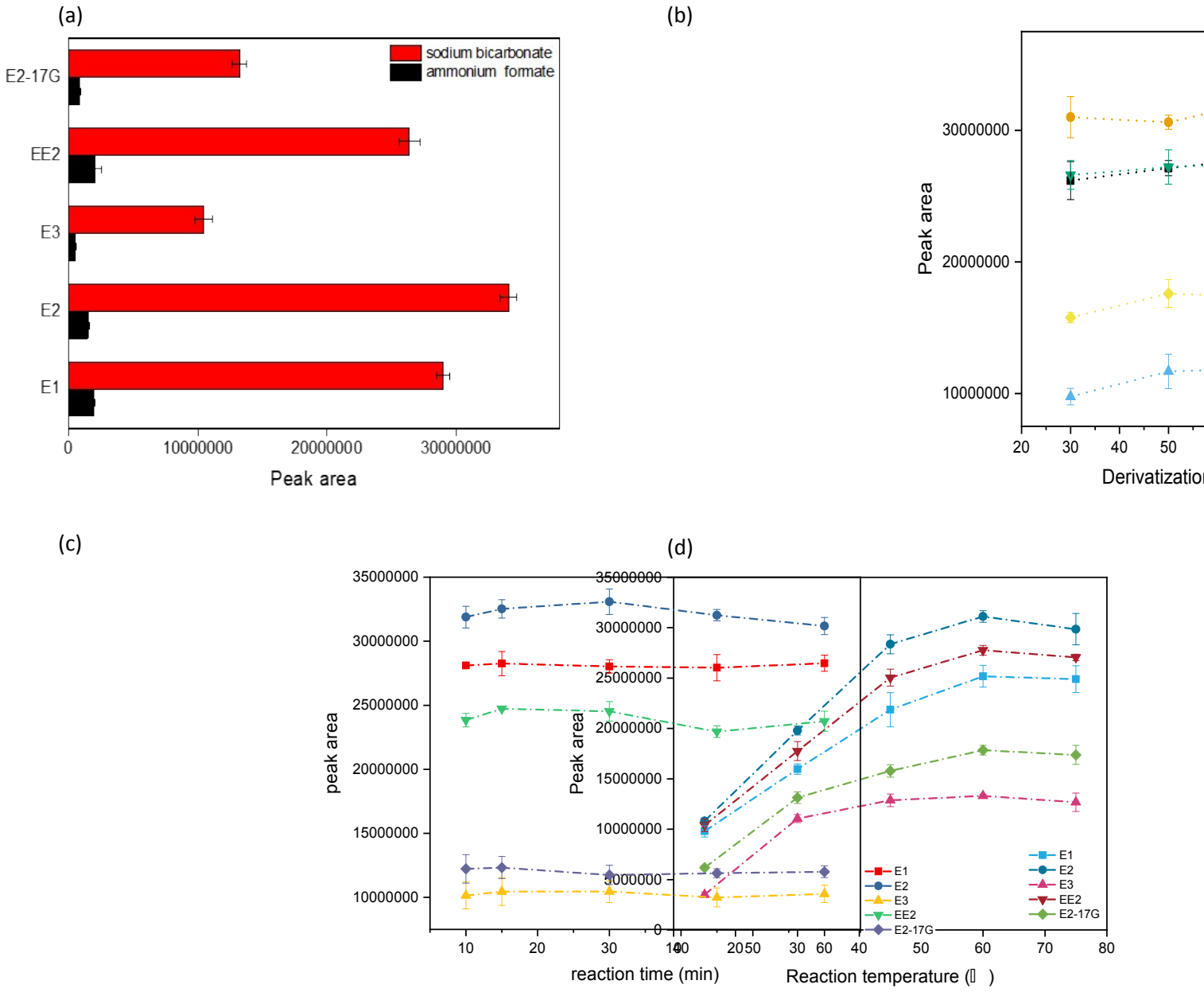


Figure S3. Optimization of derivatisation reactions by comparing estrogen derivatives yield when different effect factors were tested. a) selection of alkaline buffers; b), c) and d) optimisation of derivatisation reagent volume, reaction time and reaction temperature.

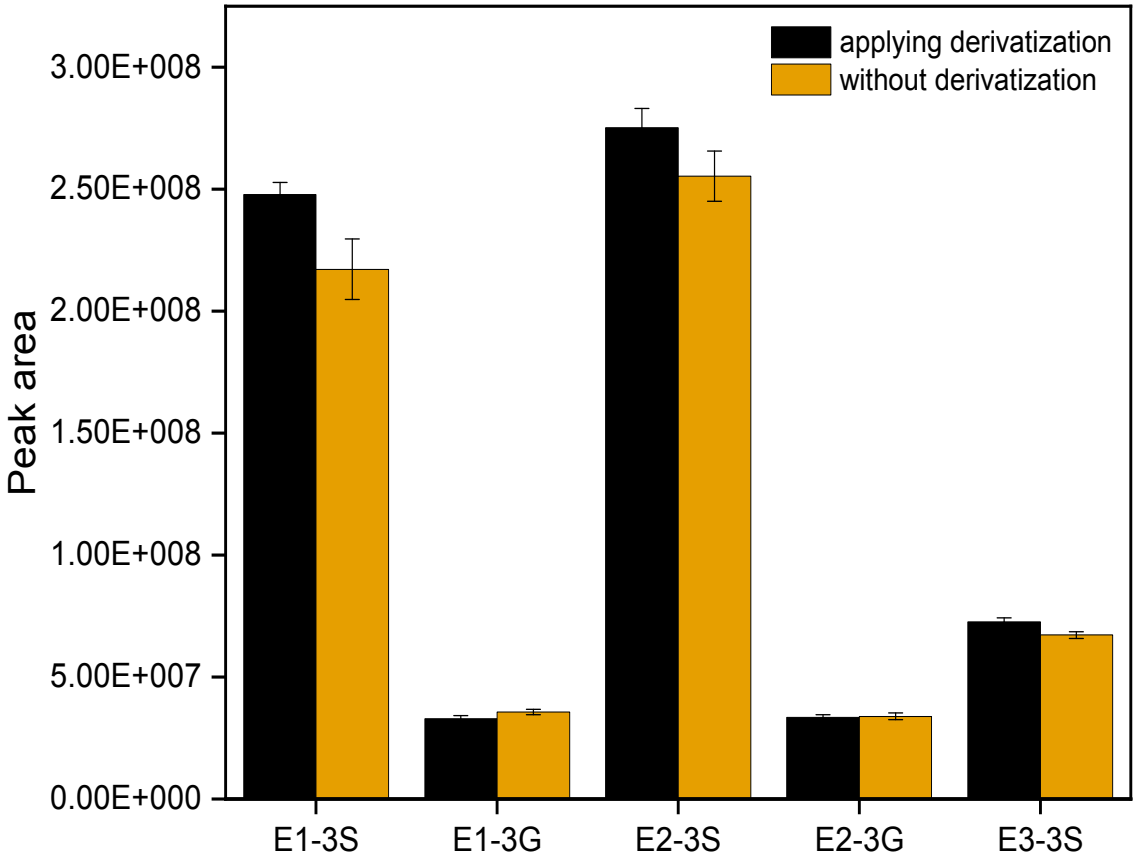


Figure S4. Stability of intact conjugated estrogens in derivatisation process (SD was expressed with error bar).

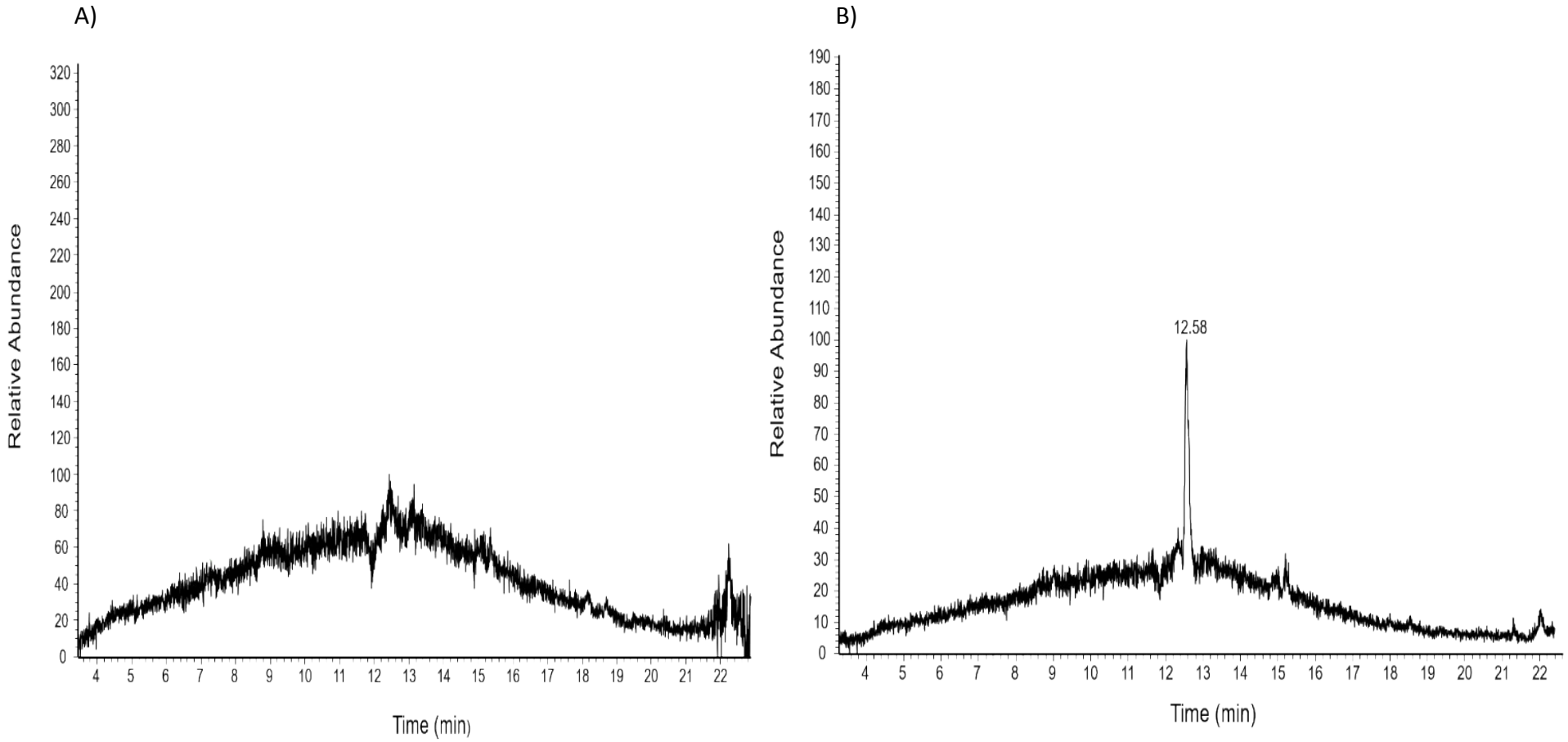


Figure S5. Reconstructed ion chromatograms of E3-DMIS at m/z 447.3 (retention time, 12.58 min) for natural surface water samples. A) non-dosed matrix sample; B) matrix sample dosed with IQL level of E3.

Table S2. Calibration curves and instrumental limits of detection and quantification for estrogen standard diluents.

Estrogens	Standard diluents				
	Linear range (μg L^{-1})	Linear slope	R^2	IDL (ng L^{-1})	IQL (ng L^{-1})
E1	0.01-1000	181.1 ± 0.92	0.9992	4.6	15.2
E2	0.01-1000	172.1 ± 0.42	0.9993	5.8	19.2
E3	0.05-1000	72.9 ± 1.36	0.9993	21.4	71.4
EE2	0.01-1000	157.2 ± 0.35	0.9996	4.7	15.6
E2-17G	0.02-1000	96.3 ± 1.68	0.9977	14.0	46.7
E1-3S	0.005-1000	321.2± 1.59	0.9998	2.5	8.5
E1-3G	0.05-1000	29.6 ± 0.31	0.9993	27.8	92.7
E2-3S	0.005-1000	460.7 ± 1.85	0.9991	1.8	5.9
E2-3G	0.05-1000	54.7 ± 0.42	0.9996	29.0	96.6
E3-3S	0.05-1000	76.9 ± 1.01	0.9995	16.8	55.8