

Occurrence of Hydroxytyrosol, Tyrosol and Their Metabolites in Italian Cheese

Danilo Giuseppe ¹, Carolina Barola ¹, Elisabetta Bucaletti ¹, Simone Moretti ¹, Fabiola Paoletti ¹, Andrea Valiani ¹, Raffaella Branciarì ²
and Roberta Galarini ^{1,*}

¹ Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Via G. Salvemini 1,
06126 Perugia, Italy; d.giusepponi@izsum.it (D.G.); c.barola@izsum.it (C.B.); e.bucaletti@izsum.it (E.B.); s.moretti@izsum.it (S.M.); f.paoletti@izsum.it (F.P.);
a.valiani@izsum.it (A.V.)

² Department of Veterinary Medicine, University of Perugia, Via San Costanzo 4, 06126 Perugia, Italy;
raffaella.branciarì@unipg.it

* Correspondence: r.galarini@izsum.it; Tel.: +39-075-343272; Fax: +39-075-3433060

Table S1 – Moisture contents and concentrations of phenylethanoids in commercial cheeses (continued)

Sample ID	Cheese type	Moisture (%)	HT-4-S ¹ ($\mu\text{g kg}^{-1}\text{ dw}$)	HT-3-S ¹ ($\mu\text{g kg}^{-1}\text{ dw}$)	T-S ¹ ($\mu\text{g kg}^{-1}\text{ dw}$)	T ¹ ($\mu\text{g kg}^{-1}\text{ dw}$)	HT ¹ ($\mu\text{g kg}^{-1}\text{ dw}$)	Sum ($\mu\text{g kg}^{-1}\text{ dw}$)
1	Ewe cheese	23.43	128	84	12	ND	ND	224
2		36.55	152	113	8.9	108	ND	382
3		38.75	188	142	8.6	116	ND	455
4		38.92	278	193	16	ND	ND	487
5		30.81	332	117	ND	ND	ND	450
6		36.06	345	153	ND	ND	ND	498
7		36.75	413	235	ND	ND	ND	648
8		17.66	204	92	ND	ND	ND	296
9		38.09	278	215	13	65	ND	570
10		28.11	164	134	9.0	ND	17	340
11		36.87	91	75	11	54	ND	231
12		40.19	295	138	ND	ND	ND	433
13		29.94	192	222	24	57	ND	496
14		32.07	213	168	7.1	ND	ND	388
15		31.47	816	263	ND	51	ND	1130
16		27.73	242	184	33	228	12	710
17		37.98	208	165	11	273	8.8	674
18		37.04	176	130	15	91	ND	412
19		33.33	585	176	ND	ND	ND	760

Table S1 – Moisture contents and concentrations of phenylethanoids in commercial cheeses

Sample ID	Cheese type	Moisture (%)	HT-4-S ¹ (µg kg ⁻¹ dw)	HT-3-S ¹ (µg kg ⁻¹ dw)	T-S ¹ (µg kg ⁻¹ dw)	T ¹ (µg kg ⁻¹ dw)	HT ¹ (µg kg ⁻¹ dw)	Sum (µg kg ⁻¹ dw)
1	Goat cheese	63.66	19	12	7.8	ND	ND	40
2		65.50	29	10	14	2296	ND	2349
3		50.82	52	23	12	1370	ND	1456
4		38.05	12	ND	9.5	74	ND	95
1	Cow cheese	40.55	22	9	15	362	ND	409
2		39.77	9.1	ND	6.2	130	ND	145
3		41.04	12	ND	12	292	ND	317
4		40.67	27	11	18	800	ND	855
5		45.73	ND	ND	28	1593	ND	1621
6		31.33	ND	ND	30	215	ND	246
7		36.57	5.2	ND	29	ND	ND	34
8		23.49	ND	ND	24	ND	ND	24
9		33.83	5.3	ND	9.9	ND	ND	15
10		30.83	ND	ND	6.5	ND	ND	6.5
11		28.92	ND	ND	ND	ND	ND	-
12		28.17	ND	ND	ND	60	ND	60
13		26.77	ND	ND	ND	ND	ND	-

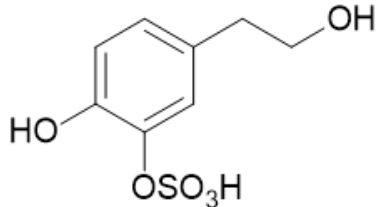
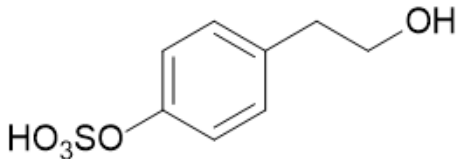
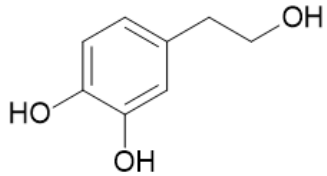
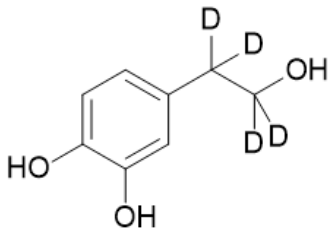
¹ND: Not Detected (< LOD); Results <LOD were placed equal to ½ LOD for statistical elaboration

Table S2 – Chromatographic conditions

Time (min)	A (%) aqueous phase (2 mM ammonium acetate)	B (%) Methanol	Curve
0	100	0	5
2	100	0	5
6	90	10	5
13	30	70	5
15	30	70	5
15.5	0	100	5
18	0	100	5
18.5	100	0	5
22	100	0	5

Table S3 - Formulas, structures and monitored ions of analytes

Analyte	Structure	Formula	RT (min)	Theoretical (<i>m/z</i>)	[M-H] ⁻ (<i>m/z</i>)	Fragment 1 (<i>m/z</i>)	Fragment 2 (<i>m/z</i>)
Tyrosol Glucuronide (T-G)		C ₁₄ H ₁₈ O ₈	6.1	314.1001	313.0929	175.0239	137.0594
Hydroxytyrosol-3- Glucuronide (HT-3-G)		C ₁₄ H ₁₈ O ₉	6.8	330.0951	329.0878	175.0237	153.0542
Hydroxytyrosol Sulphate (HT-S)		C ₈ H ₁₀ O ₆ S	6.8	234.0198	233.0125	153.0544	123.0436
HT-4-S		C ₈ H ₁₀ O ₆ S	7.6	234.0198	233.0125	153.0544	123.0436

HT-3-S		C8H10O6S	7.8	234.0198	233.0125	153.0544	123.0436
Tyrosol sulphate (T-S)		C8H10O5S	8.2	218.0249	217.0176	137.0596	119.0489
Hydroxy Tyrosol (HT)		C8H10O3	8.9	154.0629	153.0557	123.0436	108.0200
HT-d4		C8H6D4O3	8.9	158.0881	157.0808	-	-

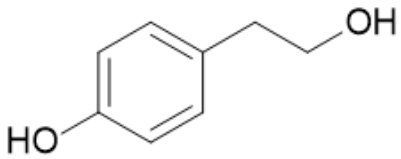
Tyrosol (T)		C ₈ H ₁₀ O ₂	10.2	138.0675	137.0608	119.0488	106.0409
-------------	---	---	------	----------	----------	----------	----------

Table S4 –Collected commercial cheese samples

Cheese	Sample ID	Producer	Moisture (%)	Classification ¹	Cheese	Sample ID	Producer (Italian region)	Moisture (%)	Classification of cheese ¹
Ewe cheese	1	Farm A (Umbria)	23.43	Very hard					
	2	Farm A (Umbria)	36.55	Semihard	Goat cheese	1	Farm I (Venetian)	63.66	Fresh /curd
	3	Farm A (Umbria)	38.75	Semihard		2	Farm J (Umbria)	65.50	Fresh/curd
	4	Farm A (Umbria)	38.92	Semihard		3	Farm J (Umbria)	50.82	Soft
	5	Farm A (Umbria)	30.81	Hard		4	Farm J (Umbria)	38.05	Semihard
	6	Farm A (Umbria)	36.06	Semihard					
	7	Farm A (Umbria)	36.75	Semihard	Cow cheese	1	Farm J (Umbria)	40.55	Soft
	8	Farm A (Umbria)	17.66	Very hard		2	Farm J (Umbria)	39.77	Semihard
	9	Farm B (Umbria)	38.09	Semihard		3	Farm K (Umbria)	41.04	Soft
	10	Farm C (Tuscany)	28.11	Hard		4	Farm A (Umbria)	40.67	Soft
	11	Farm C (Tuscany)	36.87	Semihard		5	Farm L (Umbria)	45.73	Soft
	12	Farm C (Tuscany)	40.19	Soft		6	Farm M (Venetian)	31.33	Hard
	13	Farm D (Tuscany)	29.94	Hard		7	Farm N (Piedmont)	36.57	Semihard
	14	Farm E (Tuscany)	32.07	Hard		8	Farm O (Venetian)	23.49	Very hard
	15	Farm E (Tuscany)	31.47	Hard		9	Farm P (Emilia Romagna)	33.83	Hard
	16	Farm F (Emilia Romagna)	27.73	Hard		10	Farm P (Emilia Romagna)	30.83	Hard
	17	Farm F (Emilia Romagna)	37.98	Semihard		11	Farm P (Emilia Romagna)	28.92	Hard
	18	Farm G (Sardinia)	37.04	Semihard		12	Farm P (Emilia Romagna)	28.17	Hard
	19	Farm H (Lazio)	33.33	Hard		13	Farm P (Emilia Romagna)	26.77	Hard

¹Cheese classification is based on rheological properties [46]

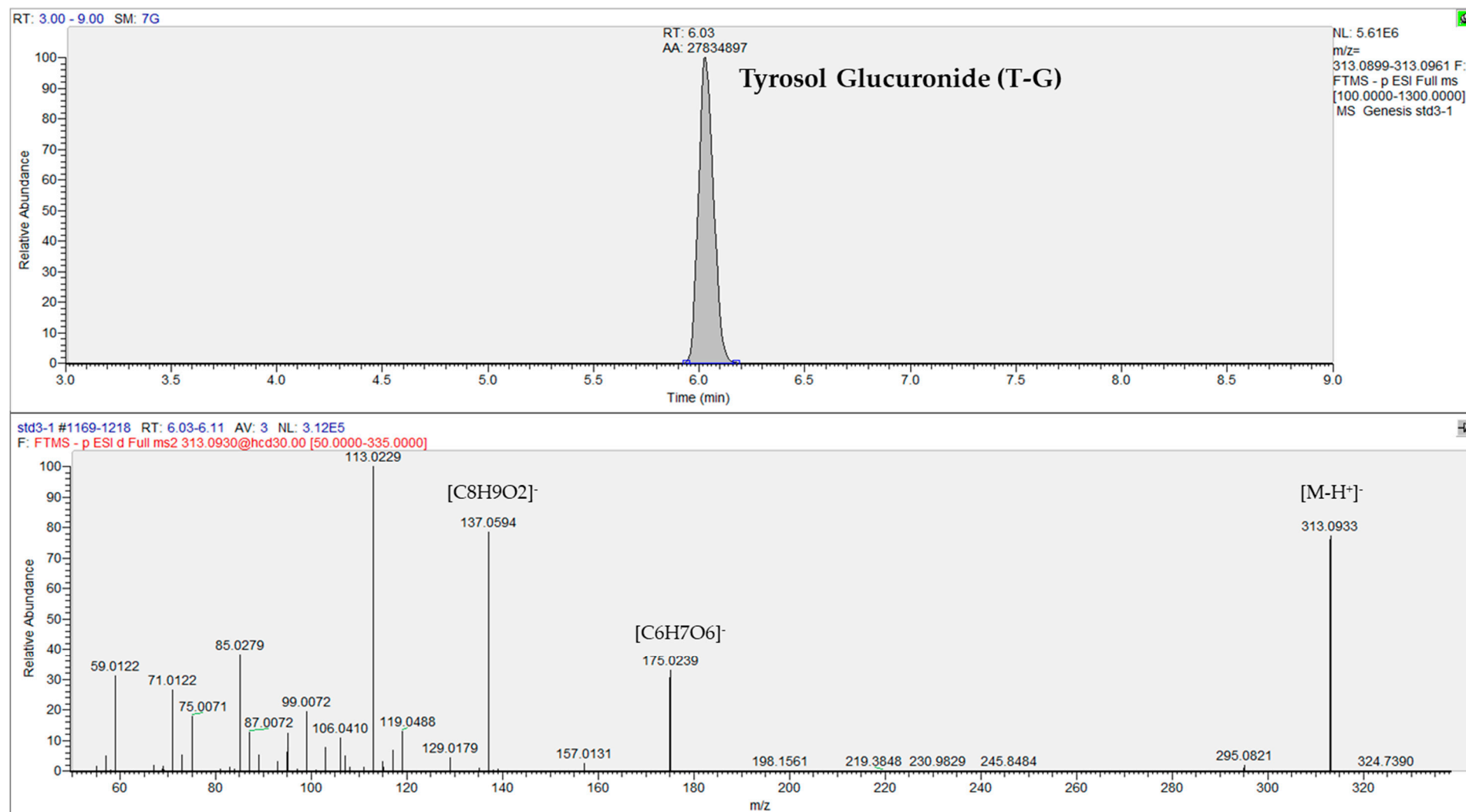


Figure S1 – LC-Q-Orbitrap chromatogram and MS² spectrum of T-G

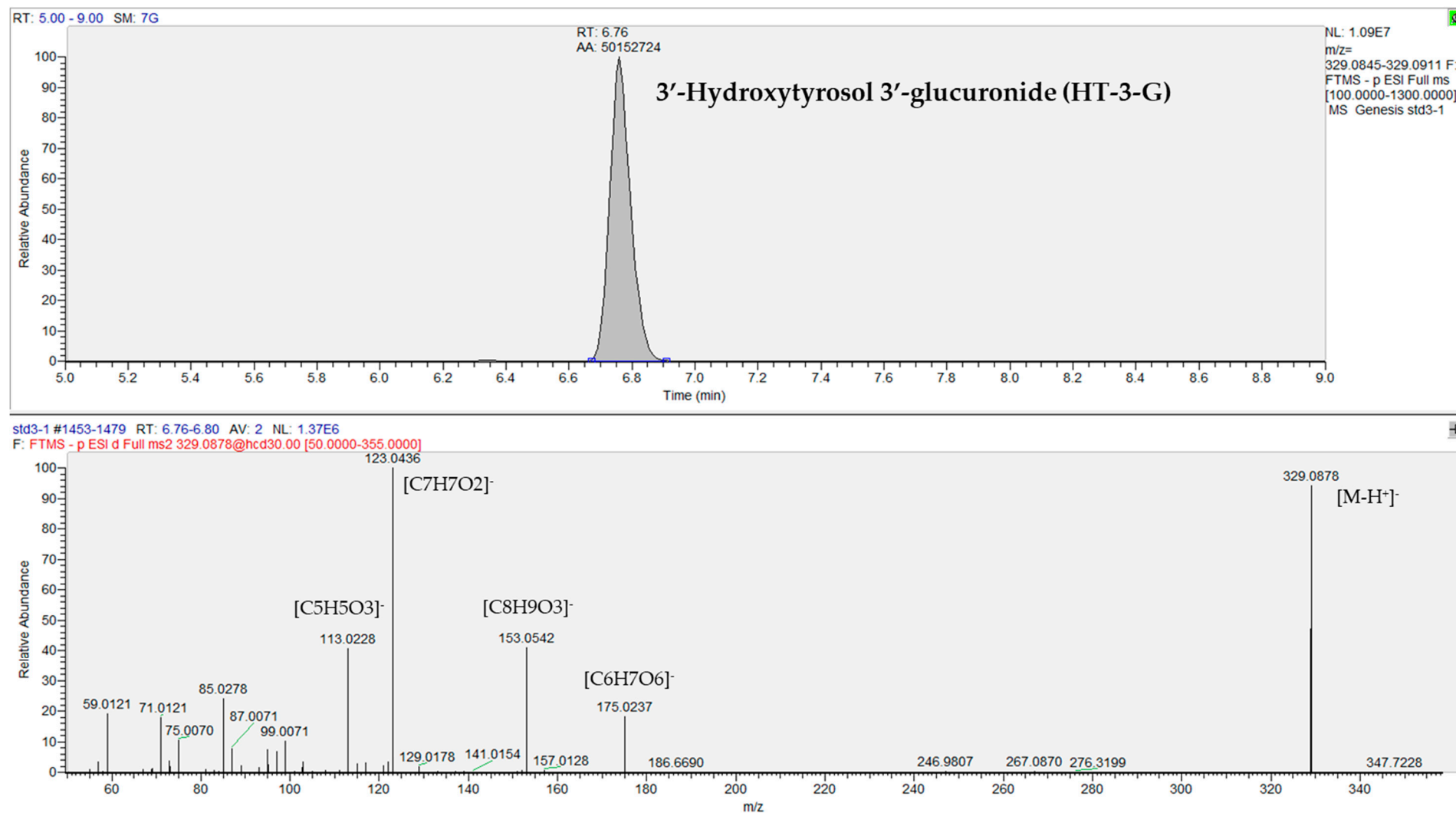


Figure S2 – LC-Q-Orbitrap chromatogram and MS² spectrum of HT-3-G

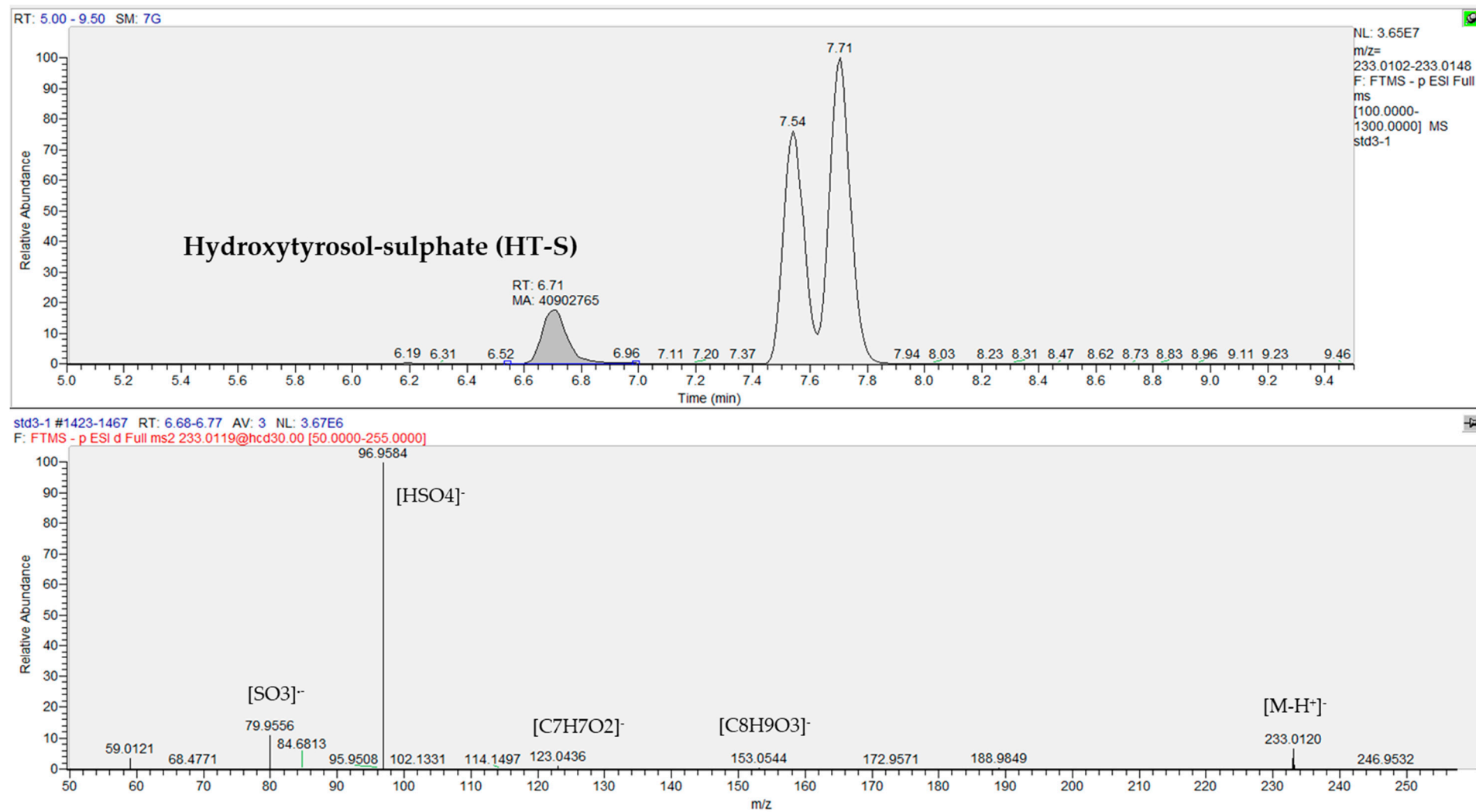


Figure S3 – LC-Q-Orbitrap chromatogram and MS² spectrum of HT-S

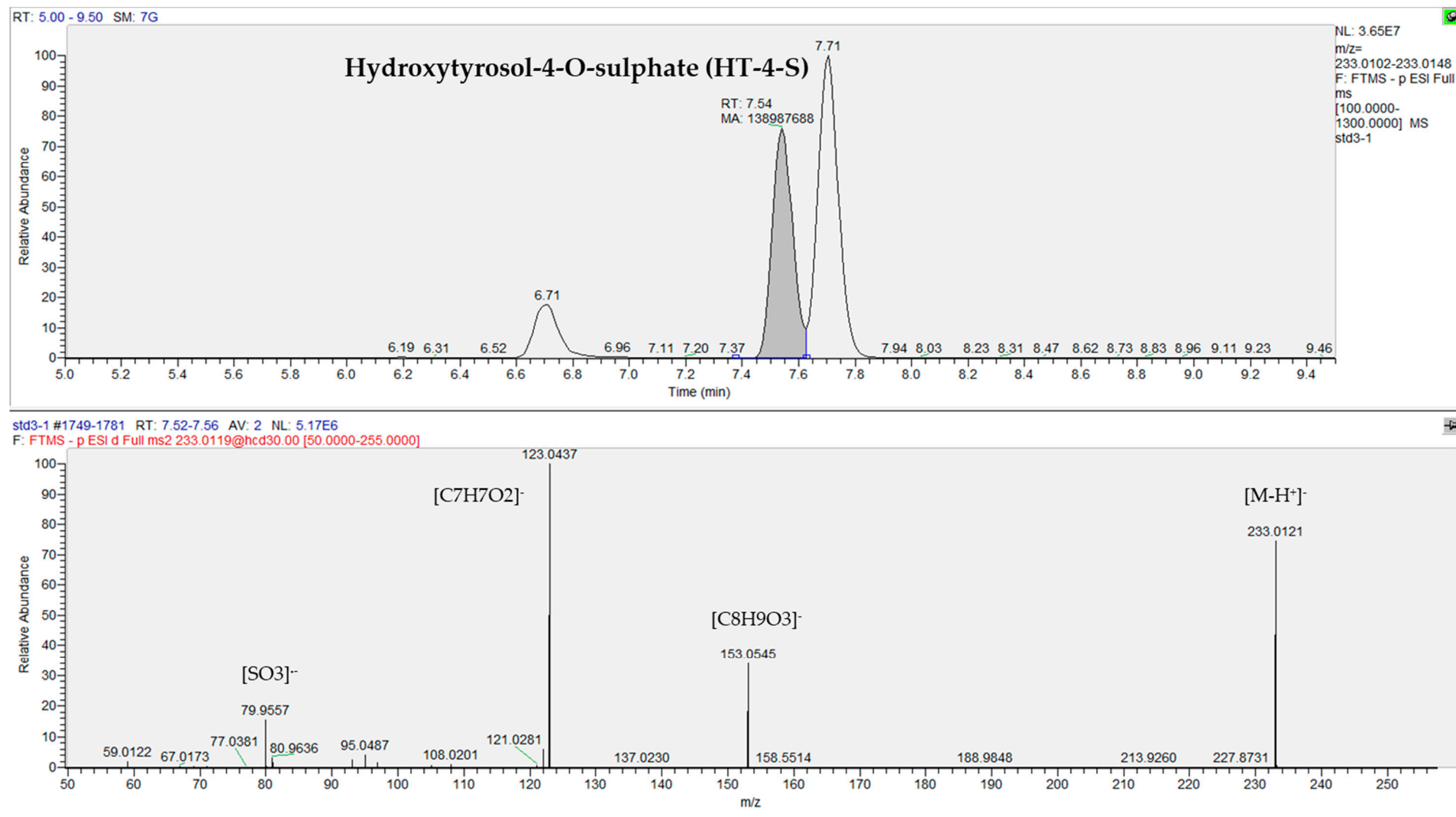


Figure S4 – LC-Q-Orbitrap chromatogram and MS² spectrum of HT-4-S

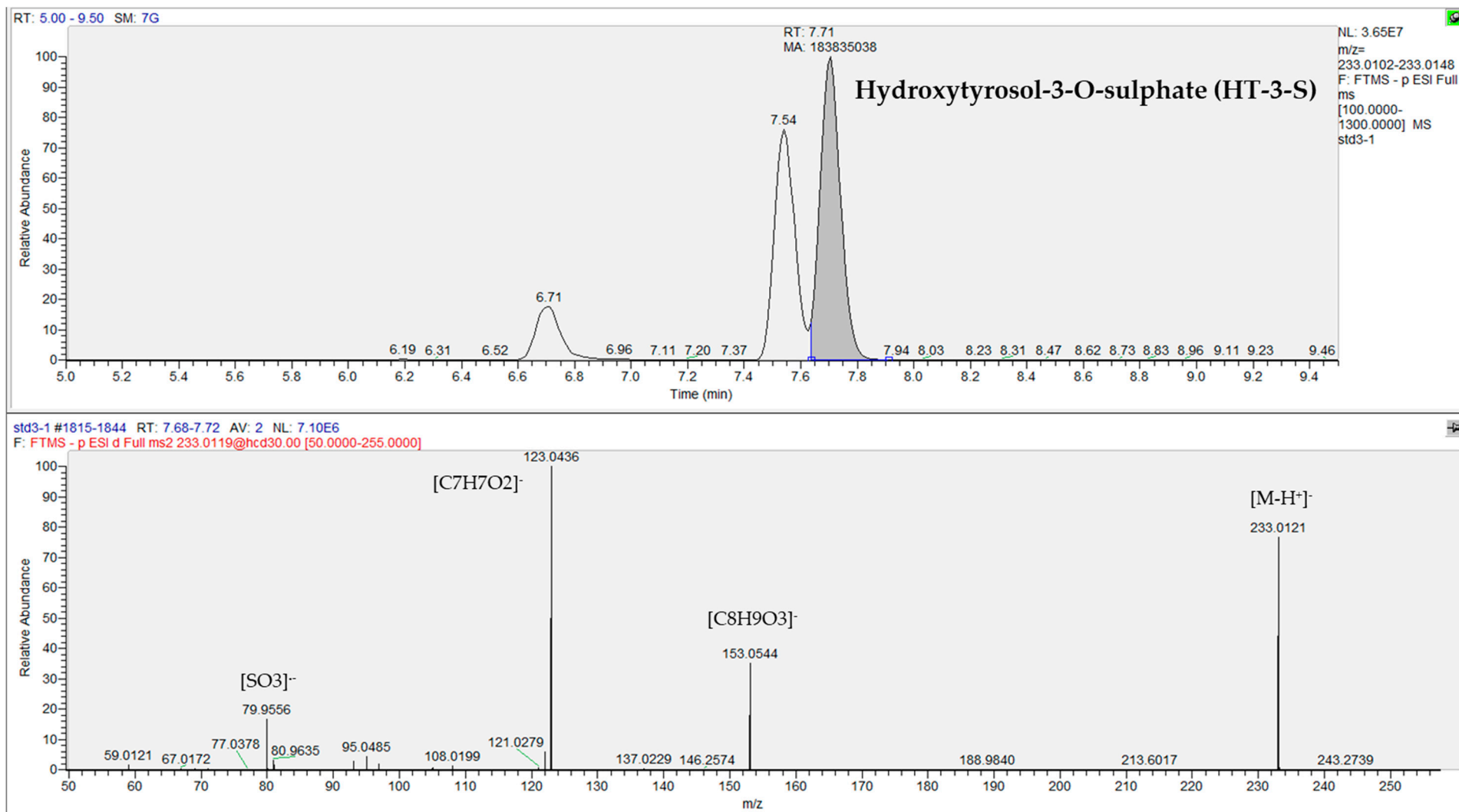


Figure S5 – LC-Q-Orbitrap chromatogram and MS² spectrum of HT-3-S

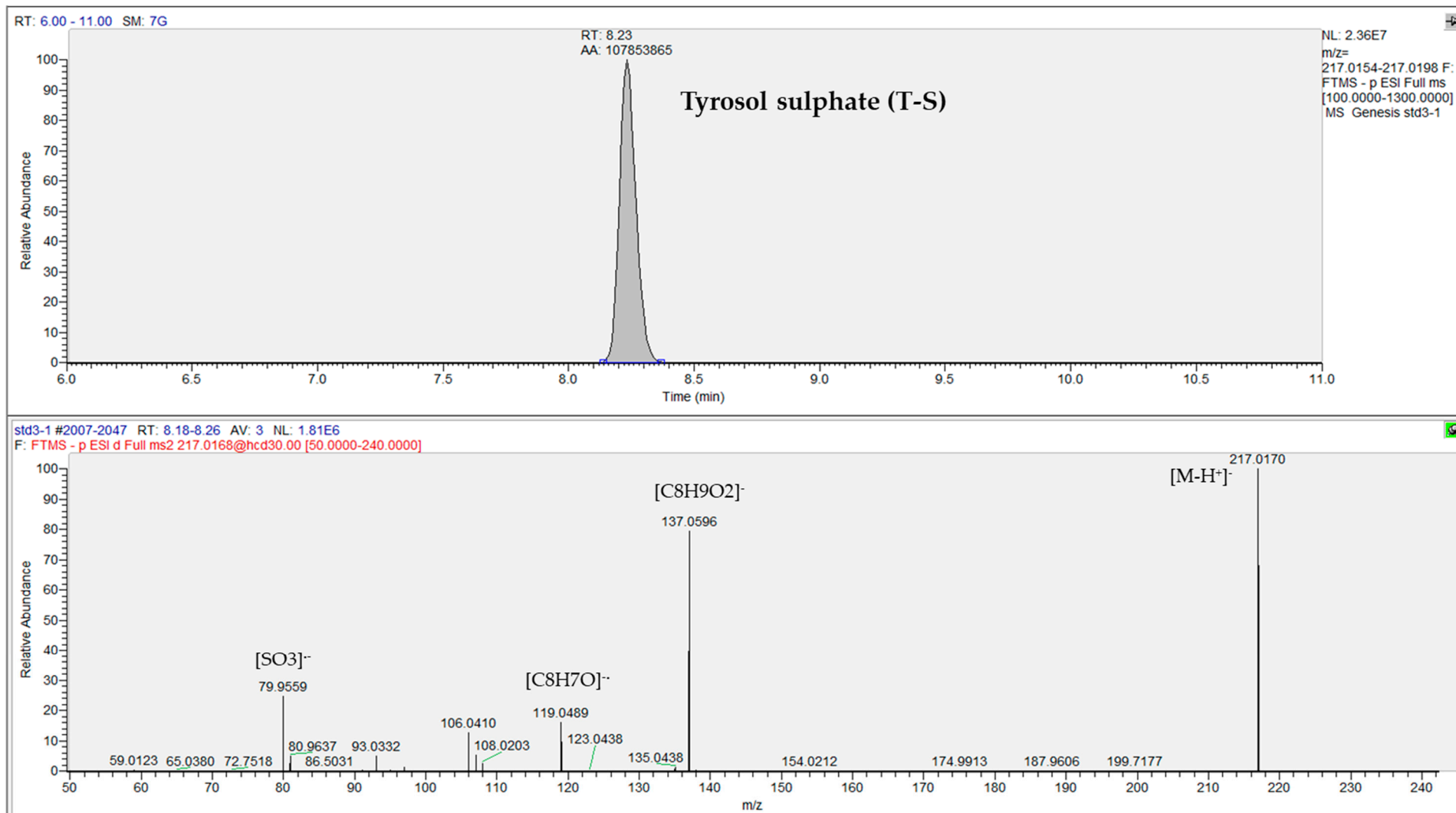


Figure S6 – LC-Q-Orbitrap chromatogram and MS² spectrum of T-S

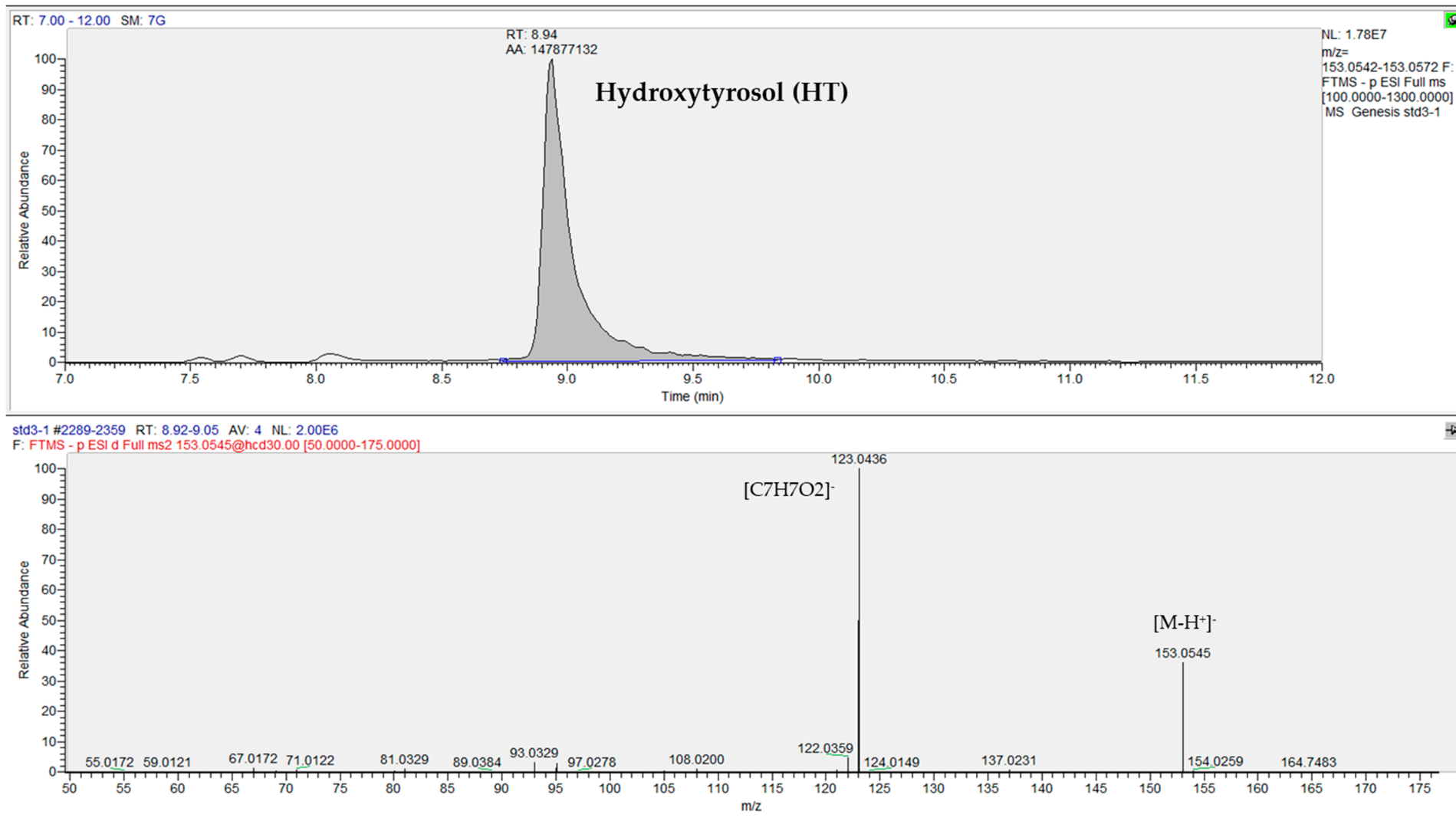


Figure S7 – LC-Q-Orbitrap chromatogram and MS² spectrum of HT

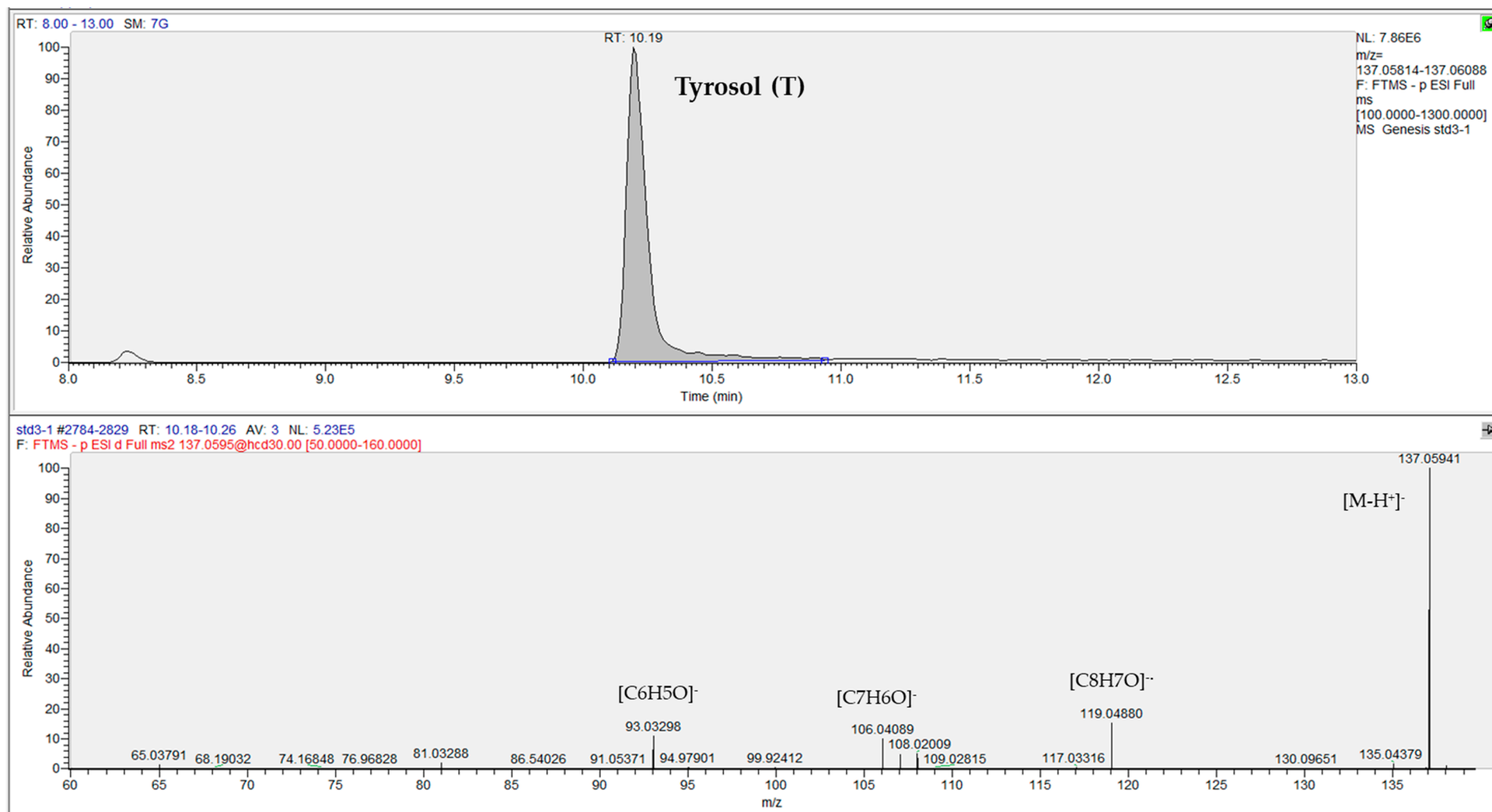


Figure S8 – LC-Q-Orbitrap chromatogram and MS² spectrum of T