

Supplementary Table 1 Food intake, water intake and body weight parameters of mice with Diphenoxylate-Induced STC in Mice treated with Ji-Chuan Decoction

	Wk	HC	STC	JCDL	JCDM	JCDH	MSP
Food intake (g)	0	3.06±0.08	3.02±0.07	3.05±0.09	3.06±0.06	3.05±0.06	3.05±0.04
	1	3.29±0.15	3.05±0.06b	0.08±0.06	3.07±0.06	3.07±0.06	3.07±0.04
	2	3.53±0.12	3.09±0.05b	3.11±0.04	3.10±0.07	3.12±0.05	3.10±0.05
	3	3.77±0.07	3.26±0.10b	3.28±0.06	3.27±0.11	3.35±0.12	3.33±0.13
	4	4.07±0.08	3.29±0.08b	3.45±0.03d	3.48±0.03d	3.61±0.06d	3.56±0.08d
Water intake (g)	0	4.3±0.67	4.1±0.55	3.5±0.35	3.8±0.45	3.±0.42	3.8±0.45
	1	3.9±0.55	3.2±1.10	3±0.14	3.04±0.16	3.08±0.23	3.16±0.17
	2	4.08±0.23	3.16±0.17a	3.16±0.17	3.12±0.18	3.08±0.11	3.12±0.11
	3	4.16±0.26	3.88±0.30	3.88±0.036	4.32±0.23	4.6±0.25d	4.56±0.17c
	4	4.56±0.17	3.36±0.17b	3.88±0.23c	4.24±0.33c	4.48±0.42d	4.64±0.26d
Body weight (g)	0	20.52±0.37	20.68±0.18	20.60±0.29	20.52±0.34	20.58±0.43	20.58±0.52
	1	21.20±0.29	20.94±0.29	20.68±0.30	20.70±0.32	20.70±0.47	20.72±0.48
	2	22.24±0.68	21.00±0.47b	20.86±0.26	20.82±0.23	20.84±0.34	20.90±0.33
	3	23.20±0.76	21.44±0.27b	21.62±0.38	21.58±0.42	22.16±0.43	22.04±0.55
	4	24.14±0.73	21.76±0.22b	22.58±0.16c	22.90±0.31d	23.64±0.42d	23.34±0.59d

HC: healthy control. STC: slow transit constipation. JCD: Ji-Chuan Decoction. JCDL: low-dosage of JCD treatment. JCDM: middle-dosage of JCD treatment. JCDH: high-dosage of JCD treatment. MSP: mosapride treatment. Data are expressed as the mean ± S.D. Compared with group HC: ^aP < 0.05, ^bP < 0.01. Compared with group STC: ^cP < 0.05, ^dP < 0.01.

**Supplementary Table 2 Differentially expressed fecal metabolites between
HC and STC groups obtained by OPLS-DA**

Metabolites	Molecular Formula	FC	P value	VIP	Trend
Prostaglandin K2	C20 H30 O5	2.336	0.0002	1.7115	up
alpha-D-Glucopyranosyl 2-O-(2-methylbutanoyl)-alpha-D-glucopyranoside	C17 H30 O12	1.6236	0.0004	2.0254	up
Genipin	C11 H14 O5	1.505	0.0007	1.9117	up
11(Z),14(Z)-Eicosadienoic acid	C20 H36 O2	0.232	0.0019	1.7209	down
Docosatrienoic acid	C22 H38 O2	0.4099	0.0022	1.3714	down
11-keto Testosterone (CRM)	C19 H26 O3	2.0437	0.0024	1.4907	up
Perillic acid	C10 H14 O2	1.5564	0.0024	2.4042	up
Corey Lactone Diol	C8 H12 O4	1.7496	0.0035	2.1169	up
19(R)-hydroxy Prostaglandin A2	C20 H30 O5	2.2814	0.0067	1.6309	up
Prostaglandin D3	C20 H30 O5	2.0414	0.0074	1.3758	up
N-Oleoyl Glycine	C20 H37 N O3	0.3856	0.0088	1.466	down
2-deoxyglucose-6-phosphate	C6 H13 O8 P	0.3166	0.0091	1.7392	down
Butylparaben	C11 H14 O3	5.5812	0.0095	1.9493	up
1,3-diazaspiro[4.5]decane-2,4-dione	C8 H12 N2 O2	1.5145	0.0119	2.0808	up
3-(4-chlorophenyl)-5-methyl-2,5-dihydro-1,2,4-oxadiazole	C9 H9 Cl N2 O	1.7725	0.0125	1.8617	up
2-(1H-1,2,3-benzotriazol-1-yl)-N-(2,3-dihydro-1H-inden-2-yl)acetamide	C17 H16 N4 O	2.5927	0.0167	1.8304	up
Tretinoin	C20 H28 O2	3.9524	0.0175	1.7536	up
3-(2-thienyl)-1,2,4-oxadiazole-5-carbohydrazide	C7 H6 N4 O2 S	1.9748	0.0176	1.9726	up
2-(3,4-dimethoxyphenyl)-N-(4-morpholinophenyl)acetamide	C20 H24 N2 O4	1.669	0.0186	1.8301	up
Salvinorin A	C23 H28 O8	1.7126	0.02	1.7739	up
Mestranol	C21 H26 O2	2.0302	0.0229	1.1873	up

N2-Acetyl- L-lysine	C8 H16 N2 O3	2.2025	0.0242	1.8209	up
(3β,5ξ,9ξ)-3,23-Dihydroxy-1-oxoolean-12-en-28-oic acid	C30 H46 O5	1.747	0.0254	1.151	up
13,14-Dihydro-15-keto-tetranor prostaglandin F1α	C16 H28 O5	2.3442	0.0262	1.962	up
19(R)-Hydroxy prostaglandin F2α	C20 H34 O6	1.8279	0.0265	1.8993	up
Nonadecanoic acid	C19 H38 O2	0.5979	0.0269	1.2708	down
N-(2,6-difluorophenyl)-2-(4-nitrophenyl)acetamide	C14 H10 F2 N2 O3	1.718	0.0276	1.9302	up
Tetrahydroaldosterone	C21 H32 O5	1.7605	0.0283	1.8933	up
1-(2,4-dihydroxyphenyl)-2-(3,5-dimethoxyphenyl)propan-1-one	C17 H18 O5	1.552	0.0289	1.4115	up
5'-Adenylic acid	C10 H14 N5 O7 P	2.1096	0.0297	1.5625	up
3,8,9-trihydroxy-10-propyl-3,4,5,8,9,10-hexahydro-2H-oxecin-2-one	C12 H20 O5	1.7455	0.0297	1.5586	up
13Z,16Z-Docosadienoic Acid	C22 H40 O2	0.4935	0.03	1.0511	down
Docosapentaenoic acid	C22 H34 O2	0.5296	0.0307	1.4519	down
4-(beta-D-Glucopyranosyloxy)-2-methylenebutanoic acid	C11 H18 O8	1.5236	0.0333	1.7616	up
Phenobarbital-d5	C12 H7 [2]H5 N2 O3	1.9811	0.035	1.1185	up
Milbemectin A3	C31 H44 O7	2.0708	0.0367	1.4054	up
N-{6-[(7-chloro-4-quinazolinyloxy]-3-pyridinyl}-4-methoxybenzamide	C21 H15 Cl N4 O3	1.6384	0.0377	1.5654	up
4-Oxoretinol	C20 H28 O2	1.8596	0.0426	1.4603	up
3-Phosphoglyceric acid	C3 H8 Na O6 P	0.3863	0.0443	1.8348	down
beta-Estradiol 17-Acetate	C20 H26 O3	1.8491	0.0462	1.2943	up
(5ξ,9ξ)-17-Hydroxykaur-15-en-19-oic acid	C20 H30 O3	3.398	0.0464	1.426	up

1-methyl-N-(4-piperidinophenyl)-1H-imidazole-4-sulfonamide	C15 H20 N4 O2 S	1.5433	0.0488	1.7637	up
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VIP, Variable Projection Importance of the First Principal Component of the PLS-DA Model. FC, fold change. n = 5 per group.

**Supplementary Table 3 Differentially expressed fecal metabolites between
STC and JCDH groups obtained by OPLS-DA**

Metabolites	Molecular Formula	FC	Pvalue	VIP	Trend
P-Coumaroyl Agmatine	C14 H20 N4 O2	0.4467	0.0000	1.8979	down
MAG (18:4)	C21 H34 O4	0.2623	0.0002	1.9282	down
(+/-)11(12)-EET	C20 H32 O3	0.5373	0.0003	1.9803	down
alpha-D-Glucopyranosyl methylbutanoyl)-alpha-D-glucopyranoside	2-O-(2- C17 H30 O12	0.5654	0.0005	1.9074	down
11-deoxy Corticosterone	C21 H30 O3	0.3385	0.0007	2.0147	down
Lipoxin B4	C20 H32 O5	0.1103	0.0007	2.1425	down
Corey Lactone Diol	C8 H12 O4	0.6068	0.0012	1.5877	down
Gibberellin A4	C19 H24 O5	0.5898	0.0018	1.4585	down
Chenodeoxycholic acid-3-beta-D-glucuronide	C30 H48 O10	0.2929	0.0019	1.8805	down
LysoPE 18:2	C23 H44 N O7 P	3.0172	0.0021	1.2412	up
2'-O-Methyluridine	C10 H14 N2 O6	0.4726	0.0024	1.7971	down
N-[2-acetyl-5-(tert-butyl)-3-thienyl]-2- (propylsulfanyl)nicotinamide	C19 H24 N2 O2 S2	0.2513	0.0024	1.6831	down
Desthiobiotin	C10 H18 N2 O3	0.6646	0.0025	1.6436	down
Hexanoic acid	C6 H12 O2	0.4659	0.0030	1.3397	down
2-Hydroxy-6-Aminopurine	C5 H5 N5 O	2.1556	0.0038	1.5054	up
1-(4-methylphenyl)-3-(2-pyridylthio)pyrrolidine- 2,5-dione	C16 H14 N2 O2 S	3.7198	0.0050	1.6043	up
Nonadecanoic acid	C19 H38 O2	2.3418	0.0052	1.7712	up
Delta-Tridecalactone	C13 H24 O2	0.6197	0.0056	1.8705	down

Glycerophospho-N-palmitoyl ethanolamine	C21 H44 N O7 P	2.8990	0.0058	1.0921	up
Myristic Acid	C14 H28 O2	0.6512	0.0059	1.0369	down
Lauric acid ethyl ester	C14 H28 O2	0.6453	0.0061	1.7908	down
Lauric acid	C12 H24 O2	0.3858	0.0063	1.6771	down
Aldosterone	C21 H28 O5	0.2196	0.0067	1.6967	down
Prostaglandin D3	C20 H30 O5	1.9494	0.0072	1.0413	up
Protectin D1	C22 H32 O4	0.0883	0.0075	1.8626	down
Tridecylic acid	C13 H26 O2	0.3468	0.0075	1.4521	down
Stachyose	C24 H42 O21	1.9492	0.0076	1.2401	up
13-HPODE	C18 H32 O4	0.1275	0.0078	1.7336	down
Lysope 18:1	C23 H46 N O7 P	2.9538	0.0081	1.1674	up
D-Sedoheptulose 7-phosphate	C7 H15 O10 P	3.4647	0.0083	1.3729	up
Tetradecanedioic acid	C14 H26 O4	3.2310	0.0100	1.5479	up
Deoxyguanosine	C10 H13 N5 O4	0.6071	0.0101	1.4625	down
LysoPE 18:0	C23 H48 N O7 P	2.8199	0.0109	1.6980	up
Prostaglandin A1 ethyl ester	C22 H36 O4	0.4558	0.0111	1.7182	down
4-Oxoretinol	C20 H28 O2	0.3953	0.0119	1.6738	down
N-{6-[(7-chloro-4-quinazolinyl)oxy]-3-pyridinyl}-4-methoxybenzamide	C21 H15 Cl N4 O3	0.5388	0.0124	1.6165	down
Oleoyl-L- α -lysophosphatidic acid	C21 H41 O7 P	1.5177	0.0128	1.1429	up
3-(4-chlorophenyl)-5-methyl-2,5-dihydro-1,2,4-oxadiazole	C9 H9 Cl N2 O	0.5878	0.0132	1.4710	down
Thymidine	C10 H14 N2 O5	2.9325	0.0133	1.4857	up
Mono(2-ethylhexyl) phthalate (MEHP)	C16 H22 O4	0.4969	0.0136	1.3782	down

4-[2-(2-oxo-1-imidazolidinyl)ethyl]- 1lambda~6~,4-thiazinane-1,1-dione	C9 H17 N3 O3 S	0.2420	0.0139	1.6795	down
Phenobarbital	C12 H12 N2 O3	0.5306	0.0139	1.8031	down
Abametapir	C12 H12 N2	0.4825	0.0141	1.7775	down
Homovanillic acid	C9 H10 O4	0.5745	0.0150	1.5457	down
7-(4-methylpiperazino)pyrimido[4,5- d]pyrimidine-2,4(1H,3H)-dithione	C11 H14 N6 S2	5.6669	0.0158	1.7262	up
Methylmalonic acid	C4 H6 O4	2.4865	0.0167	1.4745	up
N-Acetylaspartic acid	C6 H9 N O5	0.3595	0.0178	1.6362	down
Taurine	C2 H7 N O3 S	14.0811	0.0178	1.7303	up
Equol	C15 H14 O3	0.5306	0.0179	1.4802	down
(2R)-2,3-Dihydroxypropanoic acid	C3 H6 O4	2.5236	0.0185	1.3518	up
Phosphocreatine	C4 H10 N3 O5 P	0.3982	0.0189	1.4141	down
4-((5-(4-Nitrophenyl)oxazol-2- yl)amino)benzonitrile	C16 H10 N4 O3	0.0631	0.0192	1.6349	down
11-dehydro Thromboxane B2	C20 H32 O6	0.1707	0.0201	1.7004	down
4-(beta-D-Glucopyranosyloxy)-2- methylenebutanoic acid	C11 H18 O8	0.6124	0.0201	1.6265	down
Milbemectin A3	C31 H44 O7	0.2922	0.0206	1.6098	down
lipoamide	C8 H15 N O S2	0.6118	0.0212	1.1791	down
Decanoic acid	C10 H20 O2	0.2833	0.0212	1.6050	down
4-Methylphenol	C7 H8 O	2.4407	0.0237	1.4813	up
FAHFA (2:0/18:1)	C20 H36 O4	1.9788	0.0263	1.5460	up
Calcitriol	C27 H44 O3	0.5967	0.0265	1.4131	down
4-chloro-1H-indazol-3-amine	C7 H6 Cl N3	4.8623	0.0277	1.4269	up
Undecanoic acid	C11 H22 O2	0.2455	0.0280	1.4670	down

2-Hydroxyphenylacetic acid	C8 H8 O3	2.3325	0.0302	1.5107	up
Lysops 22:6	C28 H44 N O9 P	0.2491	0.0317	1.7762	down
Prostaglandin H2	C20 H32 O5	0.3163	0.0320	1.6073	down
N'-(4-chlorophenyl)-4-ethylbenzohydrazide	C15 H15 Cl N2 O	0.1483	0.0320	1.5077	down
L-Cysteinesulfinic acid	C3 H7 N O4 S	7.6690	0.0321	1.4609	up
Lysopc 20:4	C28 H50 N O7 P	0.5102	0.0356	1.4994	down
1-(1,8-dihydroxy-3,6-dimethyl-2-naphthyl)ethan-1-one	C14 H14 O3	0.0673	0.0357	1.5993	down
FAHFA (2:0/22:1)	C24 H44 O4	0.4038	0.0372	1.4694	down
PC (14:0e/2:0)	C24 H50 N O7 P	2.1885	0.0372	1.4318	up
N-[4-cyano-1-(4-fluorophenyl)-1H-pyrazol-5-yl]cyclohexanecarboxamide	C17 H17 F N4 O	0.3933	0.0378	1.6090	down
5'-Adenylic acid	C10 H14 N5 O7 P	0.4671	0.0381	1.2799	down
1,7-Dimethyluric acid	C7 H8 N4 O3	3.2898	0.0401	1.4331	up
5(S),15(S)-DiHETE	C20 H32 O4	0.3824	0.0402	1.4582	down
delta9-THC-d9	C21 H21 [2]H9 O2	2.5322	0.0402	1.4627	up
Sulfoacetic acid	C2 H4 O5 S	1.9425	0.0418	1.2433	up
Suberic acid	C8 H14 O4	0.3782	0.0425	1.2949	down
4-Hydroxyphenylacetic acid	C8 H8 O3	2.0501	0.0446	1.3926	up
Dibenzoyl Thiamine	C26 H26 N4 O4 S	0.3028	0.0478	1.5679	down
Geranylgeranyl pyrophosphate	C20 H36 O7 P2	0.4738	0.0479	1.5897	down

Bilirubin	C33 H36 N4 O6	0.1236	0.0480	1.0746	down
Sinapinic acid	C11 H12 O5	0.2081	0.0480	1.1975	down
DL-Norvaline	C5 H11 N O2	5.8327	0.0483	1.5307	up
D-(+)-Tryptophan	C11 H12 N2 O2	0.4556	0.0488	1.6309	down
(3 β ,5 ξ ,9 ξ)-3,23-Dihydroxy-1-oxoolean-12-en-28-oic acid	C30 H46 O5	1.8956	0.0497	1.0217	up

VIP, Variable Projection Importance of the First Principal Component of the PLS-DA Model. FC, fold change. n = 5 per group.

Supplementary Table 4 Different expressed stool metabolites obtained by OPLS-DA

Molecular formula	Metabolites	VIP		FC				
		HC	vs	STC	vs	HC	vs	STC
		STC		JCDH		STC		vsJCDH
C19 H38 O2	Nonadecanoic acid	1.1816		1.4790		0.5979↓a		2.3418↑d
C23 H30 N6 O4	WLH	2.0978		1.5449		0.0333↓a		29.8123↑v
C20 H28 O2	4-Oxoretinol	1.5843		1.5953		1.8596↑a		0.3953↓c
C14 H19 N O2	Normeperidine	2.2629		1.6282		0.2924↓b		3.1930↑d
C14 H28 N4 O5 S	SMK	2.0477		1.5998		0.0916↓b		11.6443↑d
C16 H14 O4	(2R)-5-hydroxy-7-methoxy-2-phenyl-3,4-dihydro-2H-1-benzopyran-4-one	1.7893		1.5140		1.7575↑b		0.4942↓c
C21 H15 Cl N4 O3	N-{6-[(7-chloro-4-quinazolinyl)oxy]-3-pyridinyl}-4-methoxybenzamide	1.6802		1.5269		1.6384↑a		0.5388↓c
C10 H14 N5 O7 P	5'-Adenylic acid	1.6488		1.2039		2.1096↑a		0.4671↓c
C8 H12 O4	Corey Lactone Diol	2.2884		1.5636		1.7496↑b		0.6068↓d
C15 H16 N2 O2	3-(dimethylamino)-1-(5-methyl-3-phenyl-4-isoxazolyl)-2-propen-1-one	1.8055		1.3464		1.8665↑a		0.5167↓c
C18 H39 N O2	Sphinganine	1.9891		1.4643		1.5514↑a		0.6347↓c
C31 H44 O7	Milbemectin A3	1.5247		1.5222		2.0708↑a		0.2922↓c
C14 H17 N3 O3	N-(3,5-dimethyl-4-isoxazolyl)-N'-(2-phenoxyethyl) urea	1.8531		1.4323		1.5850↑a		0.5963↓c
C12 H15 N O2	2-piperidinobenzoic acid	2.1340		1.6117		0.0193↓b		52.4099↑d
C9 H9 Cl N2 O	3-(4-chlorophenyl)-5-methyl-2,5-dihydro-1,2,4-oxadiazole	2.0033		1.3985		1.7725↑a		0.5878↓c

C17 H30 O12	alpha-D-Glucopyranosyl 2-O-(2-methylbutanoyl)-alpha-D-glucopyranoside	2.1923	1.8440	1.6237↑ ^b	0.5654↓ ^d
C11 H18 O8	4-(beta-D-Glucopyranosyloxy)-2-methylenebutanoic acid	1.8110	1.7904	1.5236↑ ^a	0.612352↓ ^c
C18 H17 N3 O3	1-[1,1'-biphenyl]-4-yl-2-(2-methyl-4-nitro-1H-imidazol-1-yl)-1-ethanol	1.2662	1.7532	1.7920↑ ^a	0.2886↓ ^d

VIP, the variable projection importance of the first principal component of the PLS-DA model. FC, Fold Change. HC: healthy control. STC: slow transit constipation. JCD: Ji-Chuan Decoction. JCDL: low-dosage of JCD treatment. JCDM: middle-dosage of JCD treatment. JCDH: high-dosage of JCD treatment. MSP: mosapride treatment. n=5 per group. Compared with group HC: ^aP < 0.05, ^bP < 0.01. Compared with group STC: ^cP < 0.05, ^dP < 0.01.

Supplementary Table 5 Active ingredient of Ji-Chuan Decoction

Molecular name	OB (%)	D
(20r,24r)-24,25-epoxy-3-beta-(beta-d-xylopyranosyloxy)-9,19-	40.1	0.7
(Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-	118.35	0.2
[(1S,3R)-1-[(2R)-3,3-dimethyloxiran-2-yl]-3-[(5R,8S,9S,10S,11S,14R)-11-	35.58	0.8
16β-methoxyalisol B monoacetate	32.43	0.7
1-Monolinolein	37.18	0.3
28-norolean-17-en-3-ol	35.93	0.7
alisol B	36.76	0.8
Alisol B monoacetate	35.58	0.8
alisol C monoacetate	33.06	0.8
arachidonate	45.57	0.2
Azelaic Acid	16.9	0.0
baicalein	33.52	0.2
berberine	36.86	0.7
beta-daucosterol_qt	36.91	0.7
beta-sitosterol	36.91	0.7
Choline	0.47	0.0
coptisine	30.67	0.8
Decanoic Acid	26.74	0.0
delta 7-stigmastenol	37.42	0.7
epiberberine	43.09	0.7
Ethanol	63.21	0
Heneicosanic Acid	16.14	0.2
Hesperetin	70.31	0.2
Hexadecanoic Acid	19.3	0.1
Inophyllum E	38.81	0.8
kaempferol	41.88	0.2
Marckine	37.05	0.6
Marmin	38.23	0.3
naringenin	59.29	0.2
nobiletin	61.67	0.5

Nonadecanoic Acid	17.21	0.1
paeoniflorin	53.87	0.7
palmatine	64.6	0.6
Phenylalanine	41.62	0.0
poriferasta-7,22E-dien-3beta-ol	42.98	0.7
quercetin	46.43	0.2
sitosterol	36.91	0.7
Spinasterol	42.98	0.7
Stigmasterol	43.83	0.7
suchilactone	57.52	0.5
Tuberosine A	102.67	0.3
visamminol	50.01	0.2
wogonin	30.68	0.2
Yangambin	57.53	0.8
Vitamin B12	-	-

Supplementary Figure 1 Total ion chromatograms of stool samples and QC in negative ion mode.

Specialized Traces

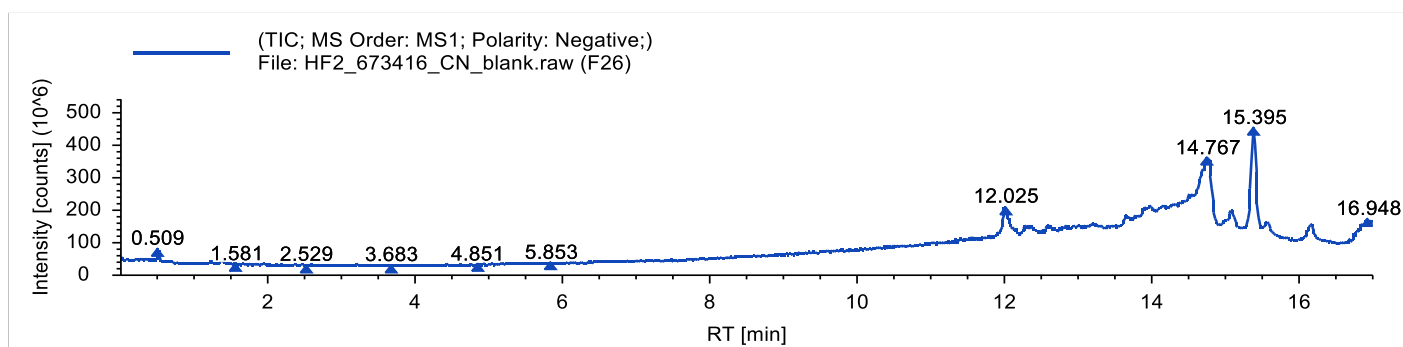
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Study File ID

F26

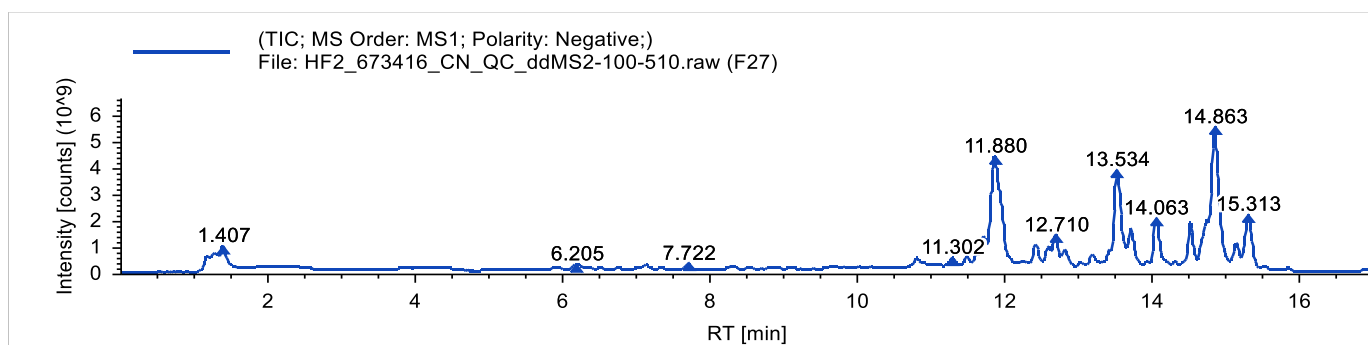
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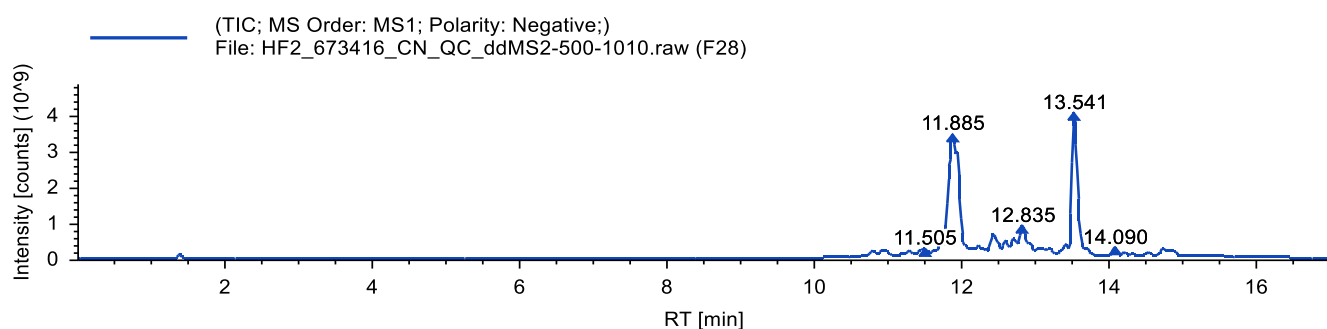
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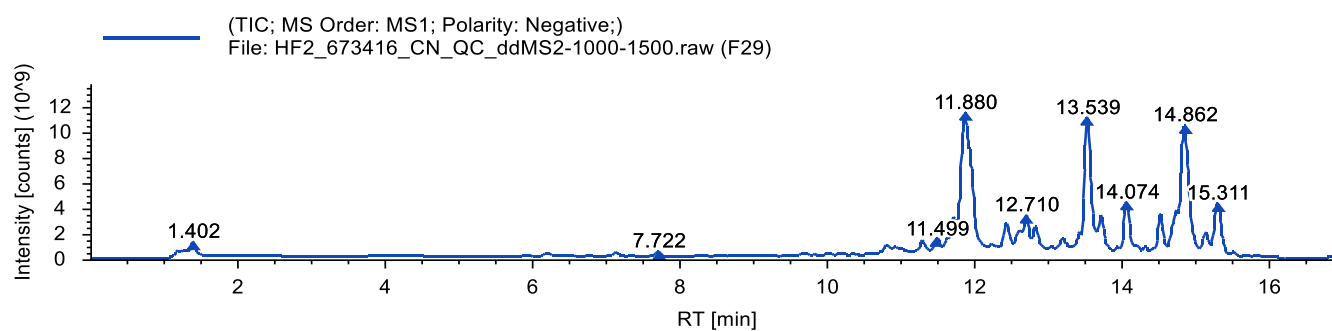
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F29

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F30

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1

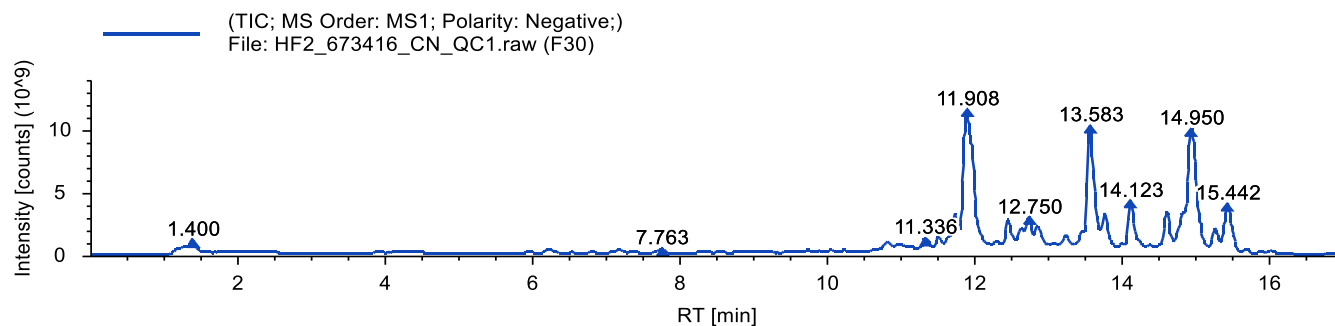
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Specialized Traces

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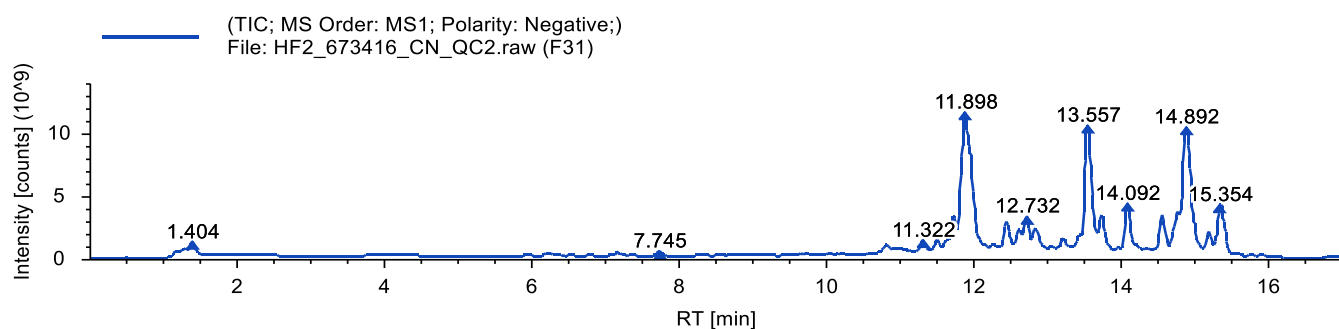
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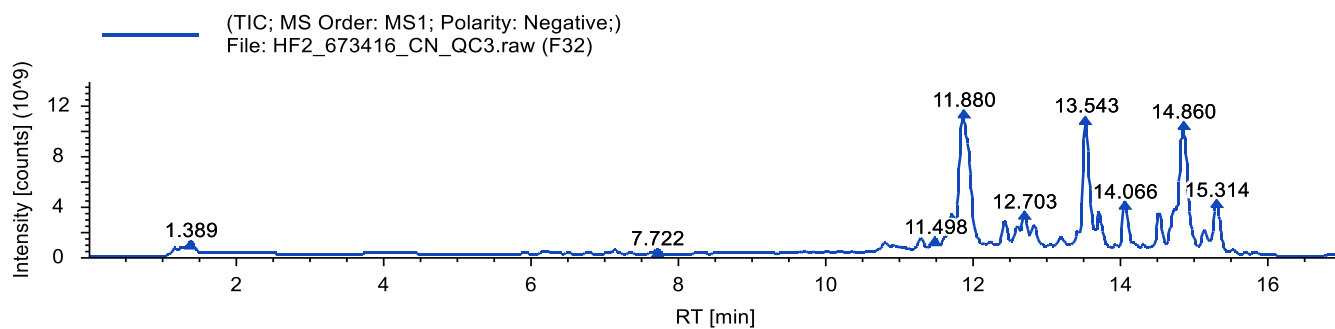
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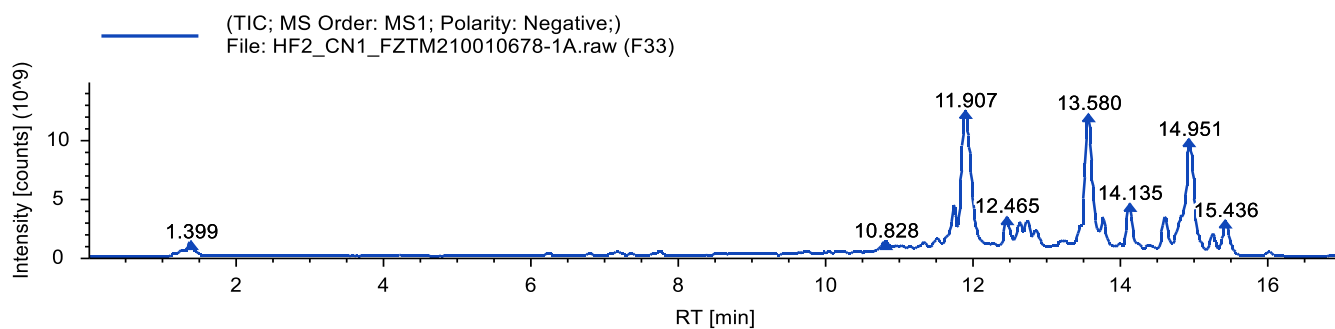
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F33

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F34

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2

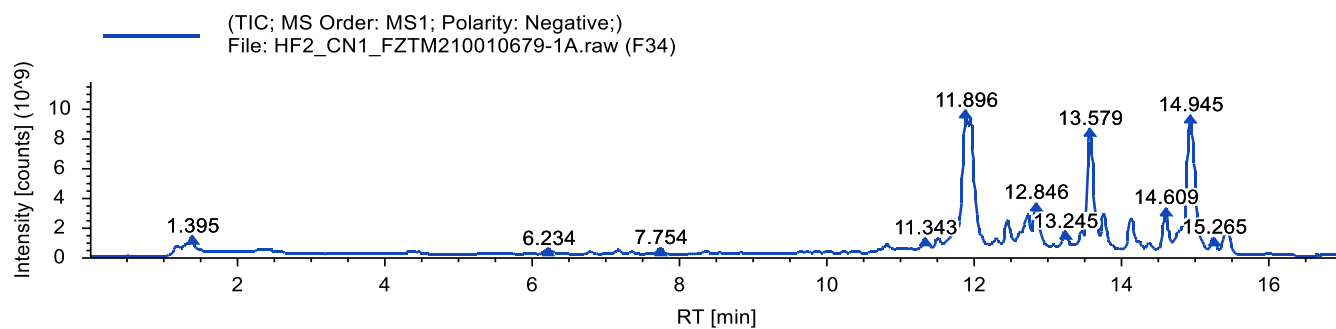
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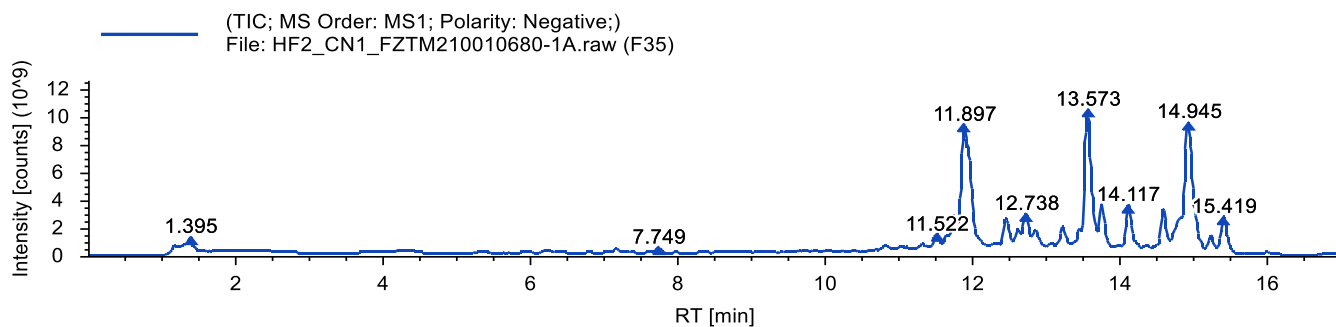
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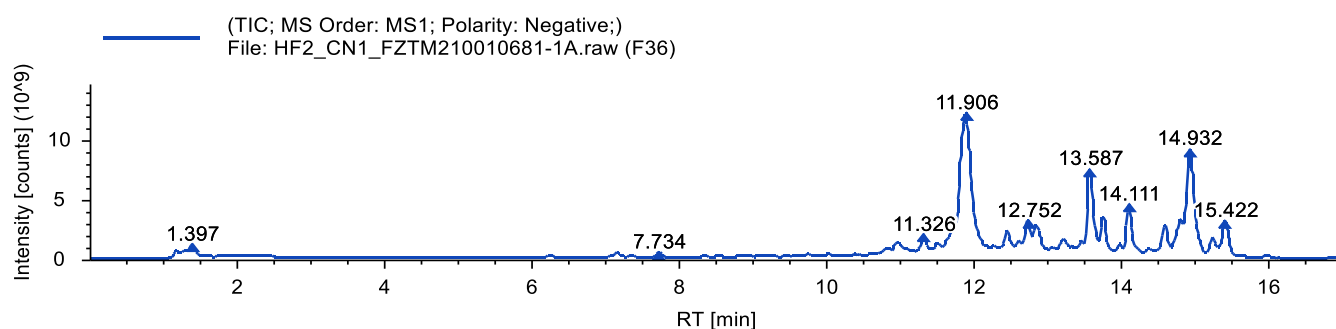
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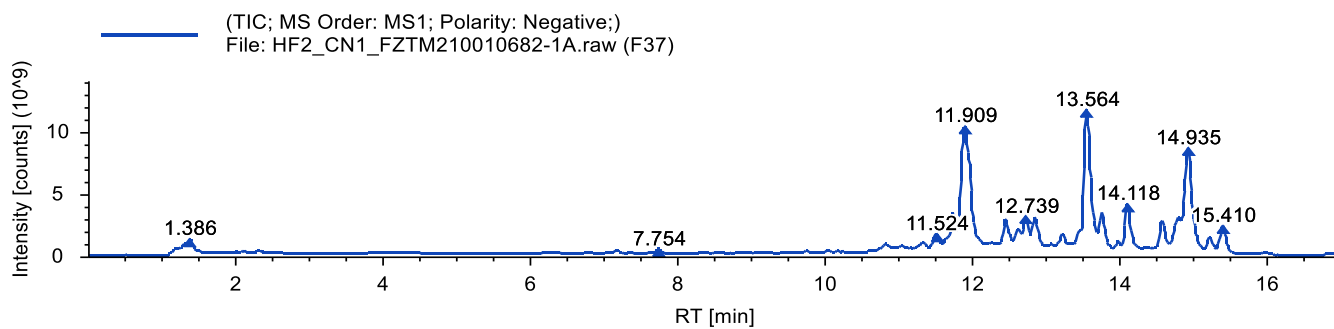
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F38

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3

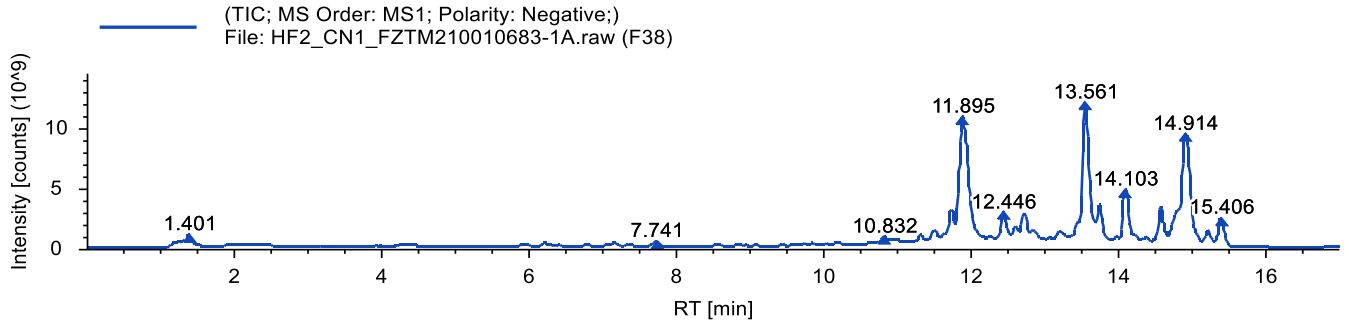
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Specialized Traces

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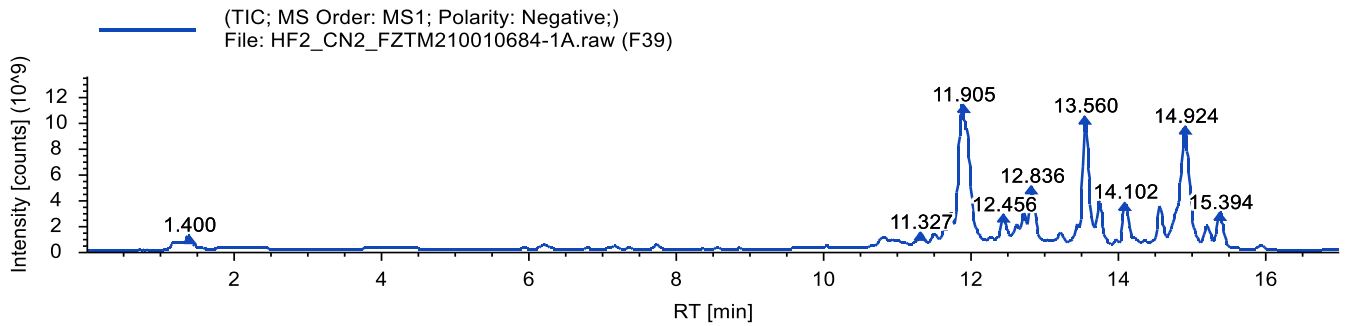
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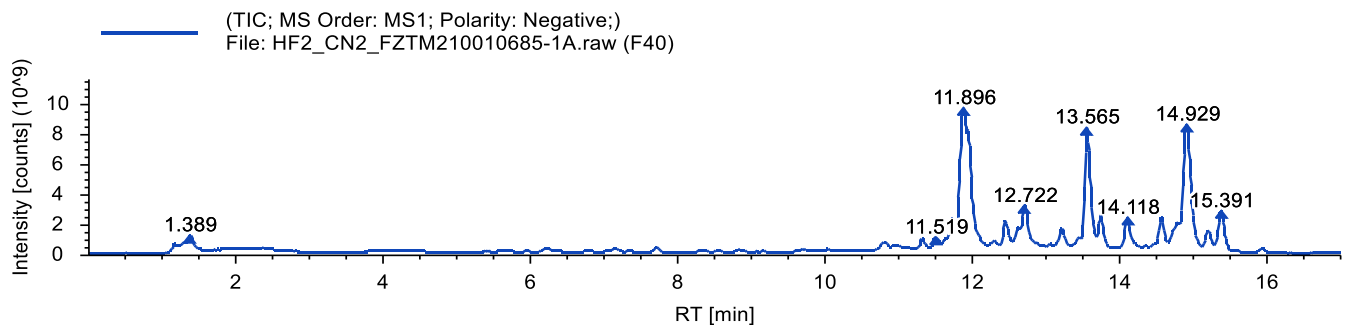
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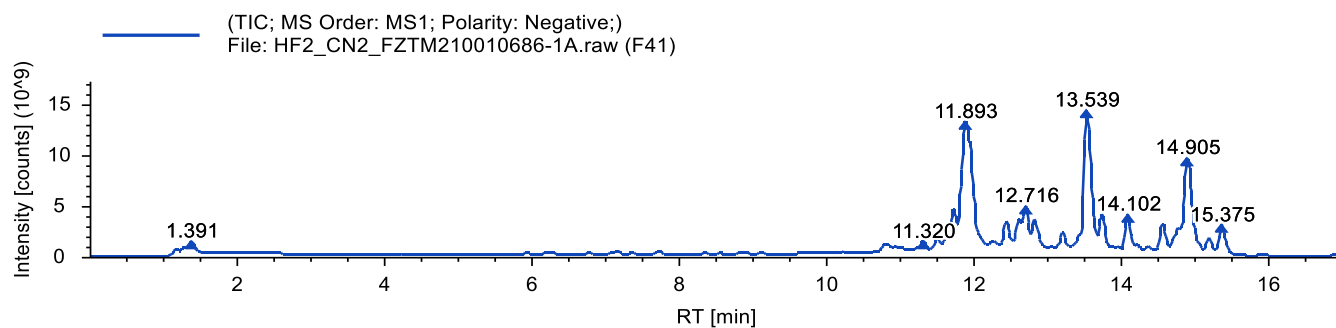
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F42

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4

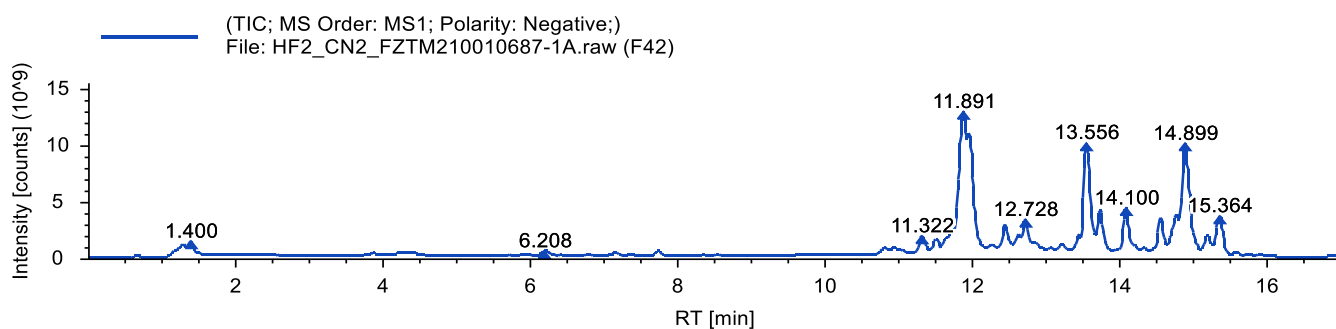
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Specialized Traces

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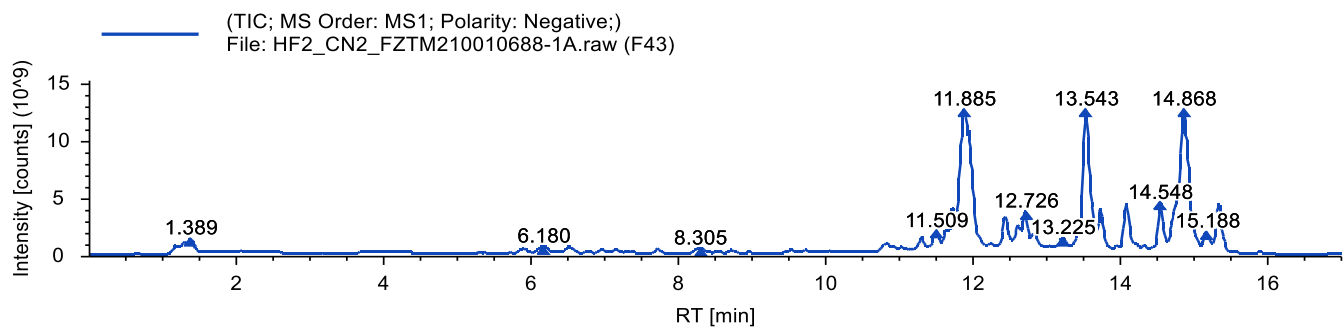
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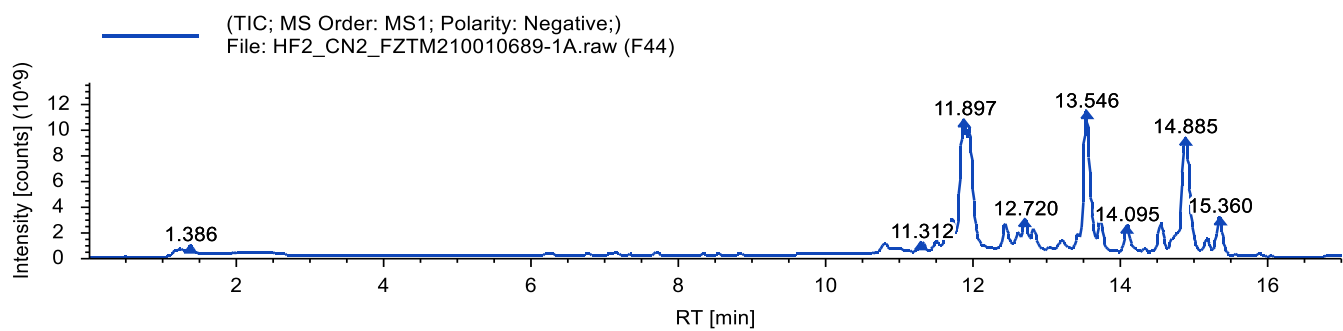
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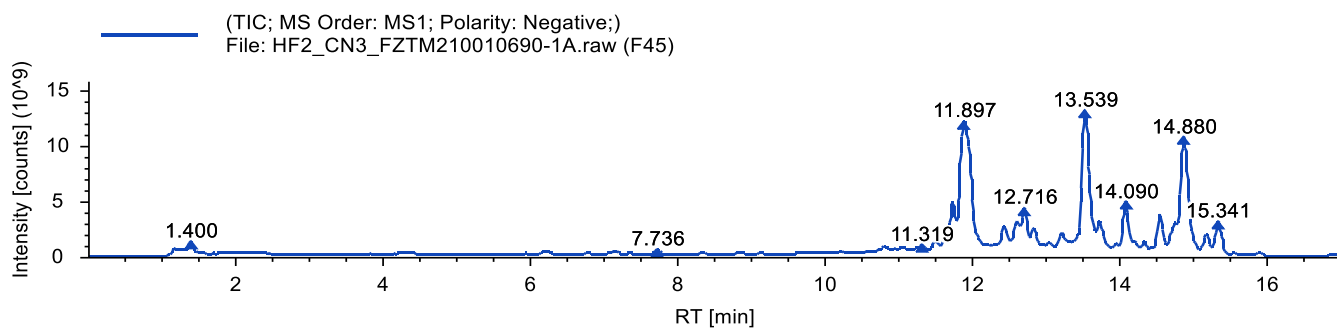
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F45

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F46

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5

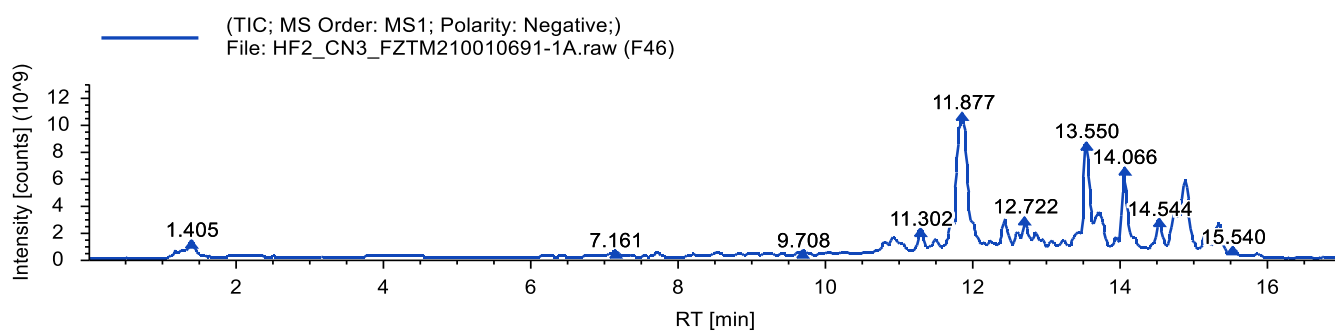
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Specialized Traces

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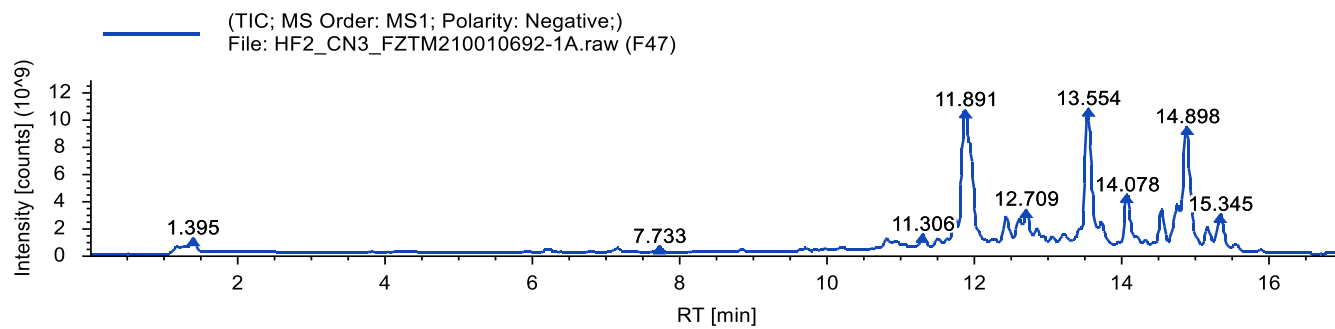
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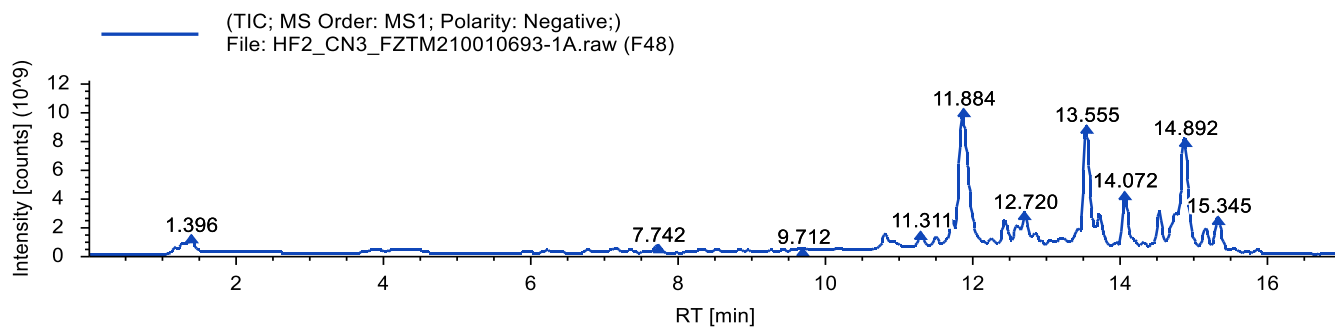
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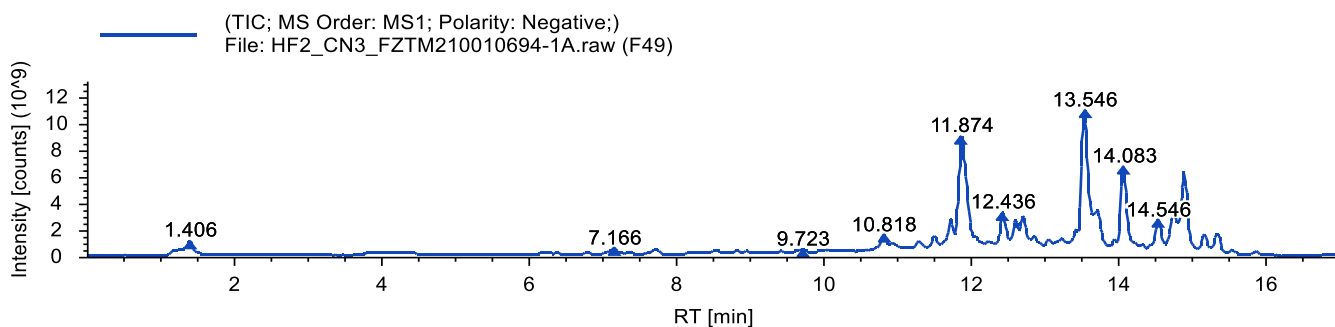
F48

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F49

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F50

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6

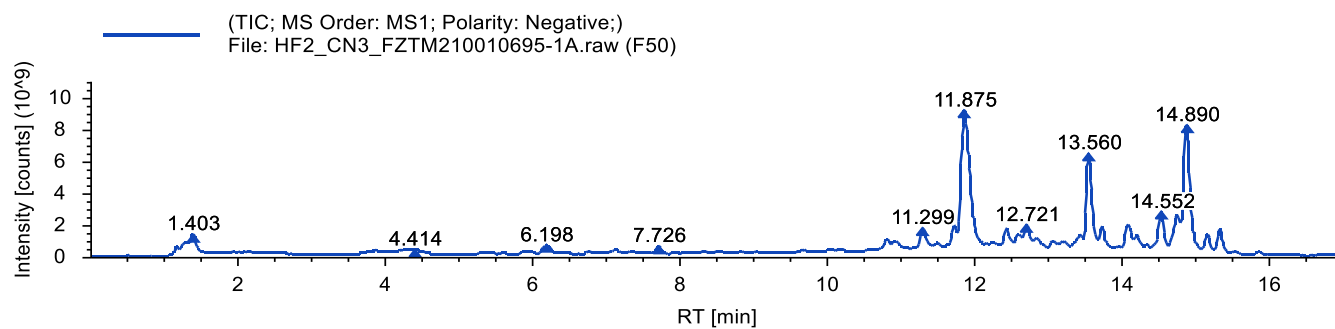
Discoverer 3.1

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Study File ID

Spectrum File



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7

Discoverer 3.1