

^2H -SIAM(1.0) Algorithm and ^2H -SIAM Pipeline

Introduction

^2H -SIAM(V1.0) is a feature list-based algorithm developed for mapping possible transformation products (TPs) of stable isotope-labeled organic matters. With the help of this algorithm, we developed a ^2H -labeled Stable Isotope Assisted Metabolomics (^2H -SIAM) pipeline which facilitate the mapping of TPs with high confidence. Additionally, our proposed ^2H -SIAM pipeline is equipped with full GUI interface which significantly lower the skill barriers of its application in research field.

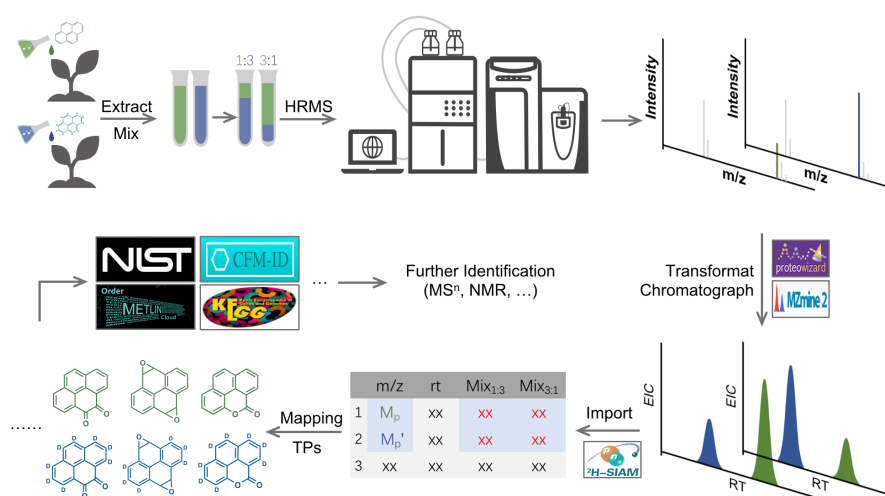


Figure 1. Proposed pipeline for ^2H -labeled Stable Isotope Assisted Metabolomics (^2H -SIAM). Firstly, both natural and ^2H -labeled parent contaminants are individually incubated in environment matrix for indicated duration; they are subsequently extracted and mixed with the ratio of 1:3 and 3:1 (Mix_{1:3} and Mix_{3:1} samples); the mixtures are determined by LC-ESI-HRMS or GC-EI-HRMS and thereafter obtained raw data are analyzed by MZmine2 to obtain feature list; then it is imported into our developed algorithm ^2H -SIAM(V1.0) to obtain possible isotopologue pairs as transformation products of contaminants for further identification.

Important tips: Although the proposed ^2H -SIAM pipeline is designed for the mapping of ^2H -labeled TPs by UPLC-ESI-HRMS, data from any other types of isotope-labeled atoms, e.g., ^{13}C , ^{15}N and ^{18}O , or any other types of LC-MS or GC-MS platform, or any other mass spectrum data processing algorithm, e.g., XCMS, MZmine2, MetaboAnalyst and MS-DIAL, are compatible for our proposed ^2H -SIAM pipeline.

^2H -SIAM pipeline

As shown in Fig. 1, a fully GUI-based pipeline is proposed, and it facilitate to lower the skill barriers of the application of ^2H -SIAM in environment study. Parent isotopologue pairs of contaminants are added into environmental matrix

respectively and incubated for indicated duration. Then, they are extracted and mixed with indicated ratio (e.g., 1:1 and 1:2, 1:3 and 3:1, 1:9 and 9:1). Here we propose a 1:3 and 3:1 ratio (Mix_{1:3} and Mix_{3:1} samples) which keep an appropriate equilibrium between accuracy and sensitivity of the mapping of isotopologue pairs.

Mixed samples are then determined by MS platform (e.g., UPLC-ESI-HRMS, GC-MS) for indicated replicates. MS raw data of Mix_{1:3} and Mix_{3:1} are transformed into .xzXML format (by ProteoWizard 3.0.20353) and imported into MZmine2 to obtain feature list information. Then, Mix_{1:3} and Mix_{3:1} data in feature list was imported into ²H-SIAM(V1.0) for TPs mapping.

Any other mass spectrum data processing algorithm, e.g., XCMS, MetaboAnalyst and MS-DIAL, are compatible if they could output feature list information as .csv format with m/z, rt and intensity (height/area). Here we propose peak height, rather than peak area, as quantitative filters, as chromatographic trailing in HPLC may significantly affect reproducibility of peak area. The key for efficient mapping TPs labeled by isotopes is to acquire high quality feature list, and then optimized parameters for the selected algorithm are necessary.

How to use ²H-SIAM(V1.0)

²H-SIAM(V1.0) accept data from any mass spectrum data processing algorithm, e.g., XCMS, MZmine2, MetaboAnalyst and MS-DIAL, in a .csv format. A feature list which is ready for use in ²H-SIAM(V1.0) is shown in Fig. 2.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	row m/z	row retentio	Mix1v3-1 Pe	Mix1v3-2 Pe	Mix1v3-3 Pe	Mix1v3-4 Pe	Mix1v3-5 Pe	Mix1v3-6 Pe	Mix3v1-1 Pe	Mix3v1-2 Pe	Mix3v1-3 Pe	Mix3v1-4 Pe	Mix3v1-5 Pe	Mix3v1-6 Pe	Peak height
2	60.0534014	9.58938719	4.74179362	6.45391255	9.49996235	8.15031894	0	0	8.23417798	0	11.8223004	7.96992713	0	5.32542415	0
3	60.2603882	15.4015725	3.32718716	3.77633901	4.86463585	2.3667658	0	7.10431636	5.11390236	2.62822296	2.58237039	5.95388082	0	4.39094929	0
4	61.029299	6.3897052	25.5668419	10.2309765	32.7835548	24.7423277	35.1519265	18.3642296	20.210568	18.3737346	14.9066701	10.9908504	6.33057595	3.19988118	0
5	63.7893214	12.7523426	3.49104261	3.25257416	4.08821879	3.79601607	0	3.45806987	3.59996878	3.26550682	5.15946469	4.40171513	4.23488707	2.37686791	0
6	64.0166295	3.9286561	3.24509871	4.05959006	5.32629956	3.59582403	5.21662408	4.92668156	4.44052733	4.69736776	3.91524268	0	3.4622314	2.92382202	0
7	65.0268543	26.0290098	9.10849282	8.76738026	0	9.81963026	16.481173	0	10.8663454	11.6007272	0	6.39194742	0	0	0
8	65.0390549	14.653747	16.3708069	17.4432704	31.9776666	19.7125718	15.9246425	16.1614012	12.2313499	13.0067821	8.46969196	8.71387247	5.90039477	8.75594725	0
9	68.0332565	19.8194984	6.1079396	9.7014614	11.6081521	9.91854263	0	10.6025977	0	6.65669658	6.68535866	0	3.84885641	0	0
10	68.0641015	21.0101205	3.07815414	4.39381677	6.61407531	4.55310059	4.86219397	4.93400814	6.3109003	7.02843879	5.9900682	6.13532276	5.33166292	2.89130879	0
11	68.3367406	6.95026493	184.919848	246.527241	389.999782	261.096265	61.5431583	75.1056291	18.1710971	0	6.73683125	17.372745	19.3350137	7.05936103	0
12	68.3367527	6.26617811	0	0	0	0	44.7394876	64.0093294	20.7385965	33.9946219	5.9994591	22.5034447	13.8021604	16.0088448	0
13	68.9979421	1.13131778	51.3359072	43.8468367	56.5358873	52.9944694	62.1072811	55.4435314	0	33.4513921	48.91871	37.3718904	22.7702258	26.4016273	0
14	68.9979425	6.25608828	0	620.528164	829.933849	0	947.460223	828.493148	578.229394	574.266183	512.088207	315.671314	164.669901	163.474783	0
15	68.9979425	7.06572336	902.876888	299.144464	376.899736	719.246023	525.515257	351.866852	390.888373	0	314.892122	281.001662	164.208013	0	0
16	69.0340218	21.7224951	35.902876	40.7026724	41.8424024	0	70.6125889	50.1389654	0	11.7382363	11.136997	12.2113835	8.66440001	7.97376675	0
17	69.0343103	13.0533758	17.9398018	18.5126207	6.22039344	18.0291045	5.66915458	13.5340592	15.1613413	18.1524166	3.40474652	13.3360139	0	10.7124771	0
18	69.0343179	11.9688248	95.3658494	133.600998	178.797392	155.506709	151.212655	149.946671	123.5861	126.08219	122.603182	113.054558	93.2380044	70.7422858	0
19	69.0706916	13.00315	46.8317291	66.4538833	105.321461	66.077959	61.2681586	65.4823237	72.478671	56.8792154	76.7834529	50.0308795	39.9168823	42.6489944	0
20	69.0706921	8.61008399	9.3955537	13.3159946	14.7193416	12.9377508	15.4686478	13.5543942	14.6576994	0	12.8417112	10.7625409	0	7.97902683	0
21	69.0706937	13.8483623	0	19.5139671	23.8329695	16.0110454	17.5748395	0	14.9891484	20.9648189	19.8120742	13.7650327	12.1187309	16.9954338	0
22	69.7892274	14.6436501	26.9229075	31.5945359	39.7277122	31.3859128	34.5786481	27.852952	20.9183735	17.9897642	18.5957872	16.5350482	15.0105866	12.5783585	0
23	69.9941724	1.24618333	5.62908835	5.84860782	5.44274968	3.70969444	10.4181444	2.84999681	4.66448832	0	7.51806046	0	0	3.66991543	0
24	70.0132496	1.12579333	19.4238414	0	0	19.5352461	42.6554493	64.1065712	72.9370425	0	0	0	0	119.746004	0
25	70.0488815	26.242138	10.2123799	0	19.0695526	14.7974089	19.5633625	15.1685952	0	13.2287941	11.4323852	8.22693152	8.49085485	0	0
26	70.0488906	28.4761048	8.49738144	8.02848651	0	9.26208015	0	16.1927019	0	0	0	6.20407735	6.22004574	5.49947389	0
27	70.0489006	25.0407292	0	9.15582622	19.1828534	9.00125175	11.2812705	11.7032875	0	0	0	0	5.38844555	0	0
28	70.0659313	27.9491221	592.893367	1244.38384	437.464952	0	9.10960211	6.9083293	0	0	0	0	0	0	0
29	70.0659339	4.17494903	8.6237307	10.3952142	12.6312442	13.1750522	16.6899481	12.7006163	0	18.3142992	0	7.79820754	5.02664311	0	0
30	70.0699285	26.4826223	8.49846829	0	19.0340294	14.8869939	15.0059572	22.2935278	19.8074331	11.1598864	7.50022466	9.92222666	6.72597028	6.45611611	0
31	70.5149582	4.30575465	3.76556464	3.7895485	0	0	5.79128876	4.77692961	0	0	0	5.23329649	0	3.17446603	0

Figure 2. Typical feature list in a .csv format used for ²H-SIAM(V1.0).

The first row is titles, including m/z, RT and intensity of Mixture1 and Mixture2 with indicated replicates (defined by customs). The first and second columns define m/z and RT (minutes) of features obtained from your data processing algorithm. **Important Notes: the sequence of the feature list should be sort by**

the increasement of m/z as shown in Fig. 2!!! The following columns are intensities (height/area) of mixture samples (e.g. Mix_{1:3} and Mix_{3:1} samples) measured by LC-MS or GC-MS. Replicates could be defined by customs.

After preparing your feature list, running ²H-SIAM(V1.0) and the following GUI interface will appear (Fig. 3). A typical setting for mapping potential TPs of pyrene by Mix_{1:3} and Mix_{3:1} samples by UPLC-ESI-HRMS (Q Exactive, Thermo) is as following: Mass tolerance 7 ppm, RT tolerance 1 min, Number of labeled atoms 3-10, Mass difference of atoms between labeled or not (Da) 1.006174, Ratio for F1, F2 and F3, 0.3333, 3 and 3, Tolerance for F1, F2 and F3, 0.3, 0.3 and 0.5.

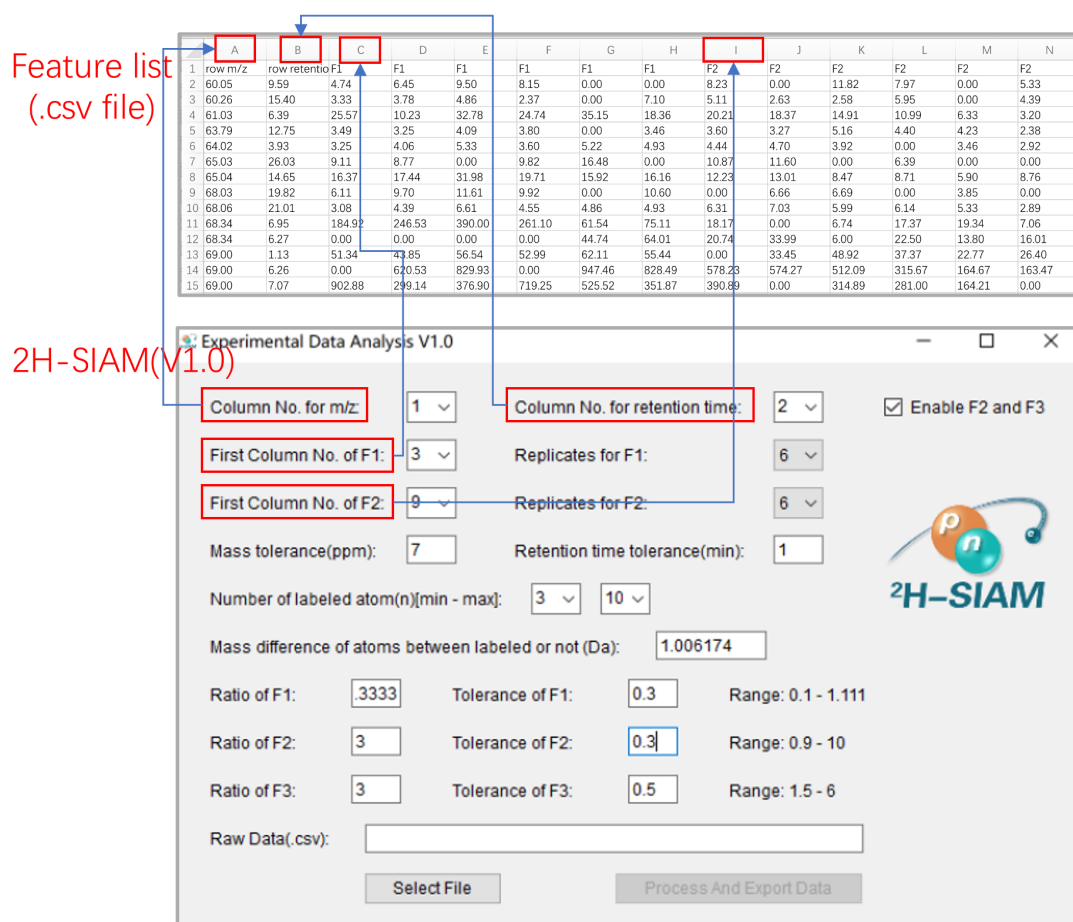


Figure 3. GUI interface of ²H-SIAM(V1.0) and its correlation with feature list (.csv).

Then select your feature list file and process, which will take several minutes depending on your PC and number of features. Our example feature list contain 4407 features, and it takes 1 minutes for TPs mapping in a PC equipped with 3.7GHz Intel Core i9. Additionally, if you have more than 10000 features, time may be significant prolonged, e.g., ~20 min. After that, a table will appear, and you could double click the table and save it as a .csv file.

When feature list (.csv format) is imported into ²H-SIAM(V1.0), it is start with qualitatively searching of isotopologue pairs with indicated isotope label, m/z and RT tolerance (Fig. 4, Step 1), and then 3 quantitative filters F₁, F₂ and F₃ are used to select potential natural TPs with a isotopologue (Step 2-4). Finally, as shown in

Fig. 5, ²H-SIAM(V1.0) will output a feature list .csv file (Fig. 4) and those features as potential TPs will be marked with numbers of potential ²H-labels. In some experiment regime, you may wish use just filter F1, and then you could remove tick before “Enable F1 and F2” .

Step 1: Paris Search					Step 2: Filter 1 (0.333, 0.11-1.1)				
	m/z	rt	Mix _{1:3}	Mix _{3:1}		m/z	rt	Mix _{1:3}	Mix _{3:1}
1	M _p	xx	xx	xx	1	M _p	xx	xx	xx
2	M _p '	xx	xx	xx	2	M _p '	xx	xx	xx
3	xx	xx	xx	xx	3	xx	xx	xx	xx

Step 4: Filter 3 (3, 1.5-6.0)					Step 3: Filter 2 (3, 0.9-10)				
	m/z	rt	Mix _{1:3}	Mix _{3:1}		m/z	rt	Mix _{1:3}	Mix _{3:1}
1	M _p	xx	xx	xx	1	M _p	xx	xx	xx
2	M _p '	xx	xx	xx	2	M _p '	xx	xx	xx
3	xx	xx	xx	xx	3	xx	xx	xx	xx

Figure 4 Algorithm for ²H-SIAM. When feature list (.csv format) is imported into ²H-SIAM(V1.0), it is start with qualitatively searching of isotopologue pairs with indicated isotope label, m/z and RT tolerance, and then 3 quantitative filters F₁, F₂ and F₃ are used to select potential natural TPs with a isotopologue.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	m/z	Retention T1A1	A2	A3	A4	A5	A6	A7	B1	B2	B3	B4	B5	B6	Mean of F1	Mean of F2	Tentative m	Filter 1	Filter 2	Filter 3			
2	219.080464	12.5380658	12.517801	17.2425444	14.6070232	10.2582801	14.2042575	12.8362985	32.9616053	36.3612127	28.5803994	29.3407491	27.0562105	26.4300584	13.6111345	30.1220259	7L	@ 226.12 10L	@ 257.1 10L	@ 275.1 10L	@ 288.4 10L	@ 296.4 10L	@ 306.60695957564
3	247.075351	12.680182	4.41478673	8.43789037	10.9845458	7.86243744	9.66229758	0	13.4008705	24.7857218	16.5046229	14.7531059	18.1720731	12.1373311	6.89365429	16.6254276	10L	@ 257.1 10L	@ 275.1 10L	@ 288.4 10L	@ 296.4 10L	@ 306.60695957564	
4	202.077737	18.3830599	929.930018	1154.28316	1404.19478	1620.18402	1381.96173	2211.81178	1885.3054	1870.17565	1732.63713	1439.1914	1318.7785	1336.49098	1742.86331	8L	@ 210.12 10L	@ 1.0175 10L	@ 3.5014 10L	@ 2.63855200407218			
5	130.05575	1.224373	5.07562871	0	8.6566126	0	7.7801226	9.0545921	7.24670339	7.3309496	6.1256342	4.6680014	0	0	0.0913137	4.2282087	10L	@ 140.1 10L	@ 3.554 10L	@ 0.9468 10L	@ 2.07224492924		
6	235.075371	18.3437622	20.6910228	30.6482933	39.8945468	40.8537994	42.9438852	40.7003629	83.0171931	91.650145	87.9897631	93.295237	71.2037363	57.8287029	35.954821	80.8283222	10L	@ 245.1 10L	@ 1.0715 10L	@ 0.9038 10L	@ 3.77328013188073		
7	104.052742	13.008035	5.0323636	6.25097905	0	6.1346346	6.92040817	6.07451746	5.91873794	9.4930381	9.24806251	9.04618265	7.56760772	7.91155212	5.0889672	8.19752896	3L	@ 107.07 3L	@ 0.541688 3L	@ 1.34611 3L	@ 1.53662347245175		
8	308.12806	12.3882778	16.820591	19.787738	31.7940131	22.7005969	26.5188657	23.8565983	23.5732339	24.8702287	25.644187	20.0757173	15.0401894	16.8981082	23.5794001	21.0541486	3L	@ 311.14 3L	@ 0.91426 3L	@ 1.22225 3L	@ 1.6819105875558		
9	91.055896	12.032117	6.32707221	7.5540211	15.5275192	8.3058218	17.5497172	10.4277676	7.6209248	9.3306603	9.19153668	7.33794148	5.4866644	4.7583346	10.9477766	7.28404184	3L	@ 94.073 3L	@ 1.02085 3L	@ 1.17798 3L	@ 1.7350682380279		
10	287.164167	14.9253836	17.496824	20.615466	33.3380604	20.615466	14.4479543	17.5717152	16.7739498	13.0701497	16.7131082	14.993022	12.1524175	12.2744359	18.0807559	14.3295138	4L	@ 291.19 4L	@ 0.52185 4L	@ 0.92420 4L	@ 1.23462176436888	@ 1.74694525710834	
11	170.117561	12.6904744	16.7435994	20.9325516	26.8097004	197.400401	227.644273	214.113122	193.228545	177.589526	175.586712	133.601566	130.324208	119.050254	211.889001	154.896802	5L	@ 175.14 5L	@ 0.99594 5L	@ 1.06945 5L	@ 1.52014282606633		
12	250.118599	12.252228	8.54815092	10.6260664	10.8627382	15.8620432	12.459613	9.42840706	63.3847229	71.5441072	63.2919856	65.7895455	54.848804	37.9520322	11.3041751	62.7993246	5L	@ 255.14 5L	@ 0.56603 5L	@ 0.90719 5L	@ 1.5924101625153	@ 1.87002808699715	
13	198.097635	11.4797963	22.8162544	28.387988	43.5407573	35.5238884	85.937867	58.6005242	230.558262	94.30247	165.678241	148.910650	34.4282719	23.5657074	45.9678332	116.240602	5L	@ 203.12 5L	@ 0.39705 5L	@ 1.87210 5L	@ 1.8649090506496		
14	405.042837	14.8338763	14.9790402	20.062071	18.6500803	19.5672397	15.7589442	14.4600798	13.6554581	12.8365118	15.9472308	15.0216638	9.22413736	11.2318484	17.2465434	12.9861417	5L	@ 410.07 5L	@ 0.90788 5L	@ 2.63995415303585			
15	159.062685	1.1453208	9.6465808	11.532378	17.7807719	13.4654031	16.0584919	14.8771767	9.8702962	8.9033904	8.45742836	0	0	13.4977635	7.01954919	5L	@ 164.09 5L	@ 0.76988 5L	@ 2.63995415303585				
16	146.011438	11.955391	5.54263575	6.9600323	9.14434072	8.5504833	8.2065011	9.09863203	4.7109547	6.44501149	6.9335644	5.42714853	4.9361962	3.26751896	7.9157141	5.9843835	5L	@ 151.04 5L	@ 0.6725 5L	@ 1.442776	@ 2.665255988872		
17	188.128151	11.6885935	28.0327611	37.5001623	52.1047747	54.3707817	61.4071864	37.7953789	54.961839	79.959482	53.472193	55.945146	71.944039	55.6436363	45.2018296	61.9937789	5L	@ 189.15 5L	@ 0.62091 5L	@ 2.62895 5L	@ 3.08100719159331		
18	305.174806	13.8444041	0	0	19.9719944	13.5394524	9.2261643	9.98819661	15.0490785	9.97148473	9.24928795	11.7221244	9.43011483	8.07599149	8.78756795	10.582947	6L	@ 311.21 6L	@ 0.47140 6L	@ 0.95129 6L	@ 1.67563784892399		
19	174.95622	1.4585897	0	14.1353879	16.2891043	0	19.3920273	16.0431044	10.9627759	12.7388529	15.5341455	9.92219931	8.86077414	8.37555006	10.9760006	11.2287163	6L	@ 180.99 6L	@ 1.0494 6L	@ 2.4794883441864			
20	244.135044	15.526467	0	14.1581255	0	24.3895239	36.0200319	33.7996284	32.3084302	18.086239	23.6072653	15.3566959	0	27.2504629	18.7940135	19.4346665	6L	@ 250.16 6L	@ 0.6359 6L	@ 1.7801166	@ 2.67637505251174		
21	283.06454	13.4258668	14.6292261	25.2050001	20.4102147	31.4694437	19.2165044	20.8672567	23.5068488	19.6219702	14.2587785	11.423837	13.1578853	9.97589162	23.4662743	15.3241412	6L	@ 289.13 6L	@ 0.70075 6L	@ 1.87212 6L	@ 0.915115347696	@ 7L	@ 3.47927796167529
22	223.169271	14.882402	17.8190119	0	0	33.3241262	23.9104444	0	0	24.4247382	19.6616888	0	15.8645271	0	12.5089421	9.99178162	7L	@ 230.21 7L	@ 0.89289 7L	@ 1.08541 7L	@ 1.5210062441174		
23	311.164969	13.733965	9.88014788	13.6859271	0	3.6160673	0	0	15.1228266	21.157976	11.9029919	11.8412087	14.2914475	13.4730683	4.4969705	14.6316047	7L	@ 319.20 7L	@ 0.33301 7L	@ 1.85020 7L	@ 1.7898554222601		
24	341.232259	12.7586157	10.819215	0	17.944326	13.7185952	13.9735706	15.606305	15.804707	15.6590863	14.2743683	15.620158	13.4204564	14.6124808	12.0899137	14.9038528	7L	@ 348.27 7L	@ 2.25294 7L	@ 2.25294 7L	@ 1.73410675321473		
25	190.133905	28.541974	27.9370159	18.020482	0	12.6880203	0	27.5507214	37.2349638	27.4497058	21.4693248	19.0247163	0	22.2454779	14.3648843	21.2373648	7L	@ 197.17 7L	@ 0.79028 7L	@ 1.86198 7L	@ 1.7627175181016		
26	211.075396	12.915026	8.721064	11.1462406	15.2418955	13.7192509	11.8738372	13.657232	12.8696918	12.2869418	9.2350382	9.3251169	8.19053228	13.4721423	10.9100894	7L	@ 218.11 7L	@ 0.75256 7L	@ 1.13637 7L	@ 1.8630711550999			
27	308.133968	12.1389	13.986249	14.914077	17.002962	15.6521727	17.7870192	13.7445885	15.196552	11.6234454	11.6705711	10.6278625	7.58100007	7.26097471	15.4941635	10.6601641	5L	@ 313.16 7L	@ 0.84147 7L	@ 1.24845 7L	@ 2.05236143735226		
28	141.018224	27.992361	0	0	7.46114731	10.954002	11.7441804	17.823137	11.980055	7.3385206	10.5281638	0	8.57683781	6.33885391	7.99708127	7.49342277	7L	@ 148.06 7L	@ 0.49650 7L	@ 0.98554 7L	@ 2.1183182794549		
29	186.07541	28.545217	28.9515759	37.9731861	60.742432	35.9621956	58.6602524	28.9692367	21.5990915	37.4123742	14.4762153	23.4438261	17.1419259	41.5239102	23.8404449	7L	@ 193.11 7L	@ 0.91541 7L	@ 1.21038 7L	@ 2.30171896458755			
30	203.085441	18.3726743	14.0452021	18.7986783	24.7427215	22.7297434	25.3399732	21.7222366	58.8320797	57.0268795	61.5161476	44.0503369	37.9701751	27.144266	21.2297162	47.756475	7L	@ 210.12 7L	@ 0.46431 7L	@ 2.95938 7L	@ 2.8332673606923		

Figure 4 Typical output .csv document list isotopologue pairs as TPs. Only data that pass indicated filters will be marked with the format “nL: XXXX”, where n refers number of labeled atoms and XXX refers to the calculated ratio.

Data availability

We offer our example data for practice of ²H-SIAM pipeline. Following data could be found from our website.

18.raw file of the experiment

FeatureListOutputFromMZmine2.csv

PyreneSoilFeatureListImportFor2HSIAM.csv

OutputFrom2H-SIAM.csv

BatchModelForC18PyrSoil1T3_3T1.xml

ProteoWizard (3.0.20353) for windows

MZmine2 (2.53) for windows

²H-SIAM(V1.0) for windows

Description of the data:

18 .raw file of the experiment, contain raw data from UPLC-ESI-HRMS (Q Exactive, Thermo) including blank, Mix1:3 and Mix3:1 with 6 replicates. It should be format to mzXML file by ProteoWizard (3.0.20353, or other version) with the following setting: Output format mzXML, Binary encoding precise 64-bit, Write index yes, Use zlib compression yes, TPP compatibility yes, Filter peakPicking vendor msLevel=1-, Filter polarity positive.

The screenshot shows the MSConvertGUI (64-bit) window. The 'List of Files' tab is selected. The 'File' field is empty, and the 'Browse' button is visible. Below the file list, the 'Output Directory' is also empty with a 'Browse' button. The 'Options' section on the left includes: 'Output format' set to 'mzXML', 'Extension' field, 'Binary encoding precise' with '64-bit' selected, 'Write index' checked, 'Use zlib compression' checked, 'TPP compatibility' checked, 'Package in gzip' unchecked, and several unchecked options for numpress and SIM. The 'Filters' section on the right shows 'Peak Picking' selected. The 'Algorithm' dropdown is set to 'Vendor (does not work for UNIFI, and it MUST be th)'. The 'MS Levels' are set to '1', 'Min SNR' is '0.1', and 'Min peak spacing' is '0.1'. A table at the bottom right lists filters: 'peakPicking' with parameter 'vendor msLevel=1-' and 'polarity' with parameter 'positive'. The 'Presets' dropdown is set to 'Generic Defaults', and the 'Files to convert in parallel' field is empty. The 'Start' button is at the bottom right.

Filter	Parameters
peakPicking	vendor msLevel=1-
polarity	positive

FeatureListOutputFromMZmine2.csv, feature list obtained by MZmine2 by batch model using the script offered here. Standard compound normalizer and export to .csv is accomplished manually. Before use, blank data should be deleted and data should be sort by the increasement of m/z. An example is uploaded with the file name PyreneSoilFeatureListImportFor2HSIAM.csv

PyreneSoilFeatureListImportFor2HSIAM.csv, typical ready for use .csv file for 2H-SIAM.

OutputFrom2H-SIAM.csv, 2H-SIAM(V1.0) analysis output file of "PyreneSoilFeatureListImportFor2HSIAM.csv" with the following setting:

Experimental Data Analysis V1.0

Column No. for m/z: 1 Column No. for retention time: 2 ☒ Enable F2 and F3

First Column No. of F1: 3 Replicates for F1: 6

First Column No. of F2: 9 Replicates for F2: 6

Mass tolerance(ppm): 7 Retention time tolerance(min): 1

Number of labeled atom(n)(min - max): 3 10

Mass difference of atoms between labeled or not (Da): 1.006174

Ratio of F1: .3333 Tolerance of F1: 0.3 Range: 0.1 - 1.111

Ratio of F2: 3 Tolerance of F2: 0.3 Range: 0.9 - 10

Ratio of F3: 3 Tolerance of F3: 0.5 Range: 1.5 - 6

Raw Data(.csv):

2H-SIAM

BatchModelForC18PyrSoil1T3_3T1.xml, MZmine2 analysis script for example data. Additionally, Standard compound normalizer was accomplished manually as following setting: Nearest standard, Peak measurement type peak height, m/z vs RT balance 1, Standard compounds m/z 188.1405 at 16.14 (anthracene-*d*10). Then, .csv could be exported with m/z, rt and peak height.

ProteoWizard (3.0.20353), windows software for MS raw data format.

MZmine2 (2.53), windows software for MS data analysis.

2H-SIAM(V1.0), windows software for isotope-label metabolomics.

Description of the example experiment:

pyrene and pyrene-*d*10 were dissolved in acetone (ACE) and mixed respectively with one quarter of blank soil. After solvent evaporation, they were mixed with the rest unspiked soils to obtain 100 ppm pyrene and pyrene-*d*10 contaminated soil respectively. Then, blank and contaminated soils were placed in room temperature for 60 days and watered weekly to keep wet. After 60 days incubation, soil samples from blank, pyrene and pyrene-*d*10 treatment were harvested and air dried at 60 °C until to constant weight. 1 gram of contaminated soils were extracted by 10 mL of 1:3 acetone and Hexane by microwave extraction, and anhydrous NaSO₄ were used to remove residual water. After that solvent was replaced by acetonitrile by solvent exchange, and extracts were concentrated to 1 mL under nitrogen flow.

Extracts from pyrene and pyrene-*d*10 were mixed with the ratio of 1:3 and 3:1 to obtain Mix_{1:3} and Mix_{3:1} samples. Anthracene-*d*10 were added into blank and mixed samples as internal standard. Then, blank and mixed samples were subjected to UPLC-ESI-HRMS analysis (6 replicates).

5 µL of samples was injected into UPLC-ESI-HRMS system. UPLC solvents were A, water in 0.1% formic acid, and B, acetonitrile with 0.1% formic acid. UPLC were performed at 1 mL/min at 25 °C with the following linear gradient (minutes, %B): 0, 5%; 4, 5%; 8, 95%; 26, 95%; 28, 5%; 30, 5%.

Mass spectrometry was detected via profile mode (scan range, m/z 60–900) with a resolving power of 140 000 fwhm (at m/z 200) and automatic gain control setting of 3×10^6 with a maximum injection time of 200 ms. The heated electrospray ionization (ESI) source was operated using the following settings: sheath gas flow rate, 40 au; auxiliary gas flow rate, 20 au; spray voltage, 3.8 kV; capillary temperature, 325 °C.