

Poster Reprint

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Identification of Anthropogenic Compounds in Stream Waters Using Non-target Strategies by HRMS

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Introduction

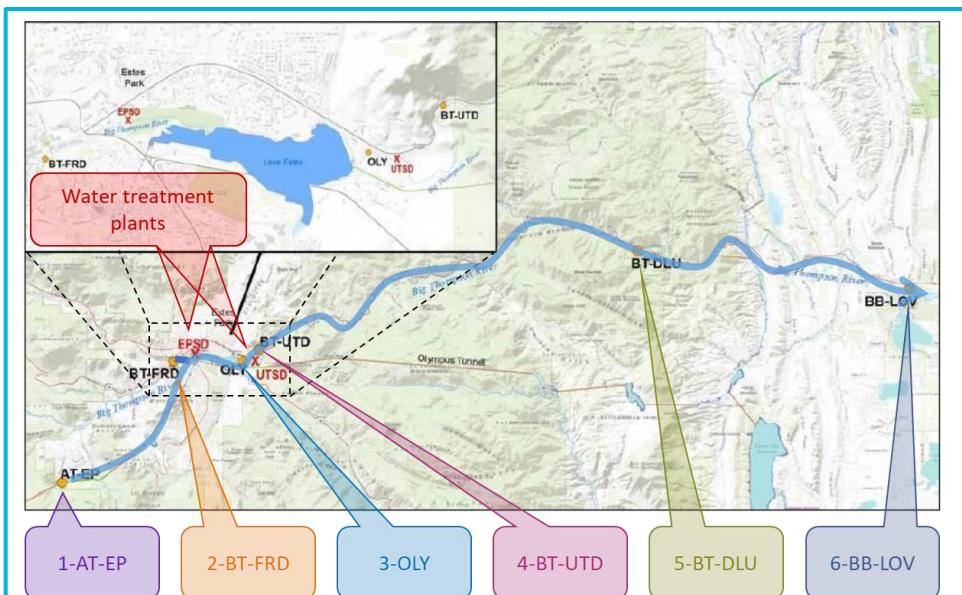
Surface waters are environmentally impacted by wastewater sources. In this work, pristine upstream water sources were compared to downstream locations to find anthropogenic compounds.

High resolution mass spectrometry using an LC/Q-TOF MS instrument was used to measure the maximum number of potential contaminants.

The main goal of this study was to identify as many compounds as possible using a combination of tools including: retention time and accurate mass databases, NIST Tandem MS/MS libraries, SIRIUS and CSI:FingerID.

Study Design

- Water samples were analyzed from 6 locations over a 5-year period along the Big Thompson River near Rocky Mountain National Park and Estes Park.
- Sample sites comprised: pristine mountain streams, used as controls, and downstream locations impacted by urban areas and wastewater.



Data Processing

- Agilent MassHunter Explorer was used for non-targeted data extraction and analysis of all the samples.
- Agilent ChemVista Library Manager was used for storage and management of in-house spectral libraries

Experimental

Sample Preparation

- Extract 100 mL of water on Oasis HLB cartridge (200mg) using Automated SPE system.
- Elute with 6 mL of MeOH.
- Nitrogen dry to 0.5 mL final volume.
- Inject 20 μ L on LC/Q-TOF MS.



Automated Solid-phase extraction (Gilson GX-271 ASPEC)



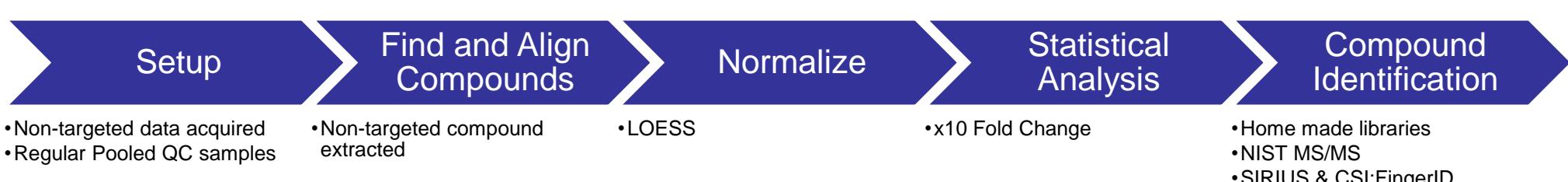
Agilent 1290 Infinity II LC coupled to a 6546 LC/Q-TOF MS

Instrument conditions

- Reverse phase chromatography (C8 column).
- Data independent (All Ions MS/MS) and data dependent Iterative MS/MS acquisition
- Positive ion electrospray.

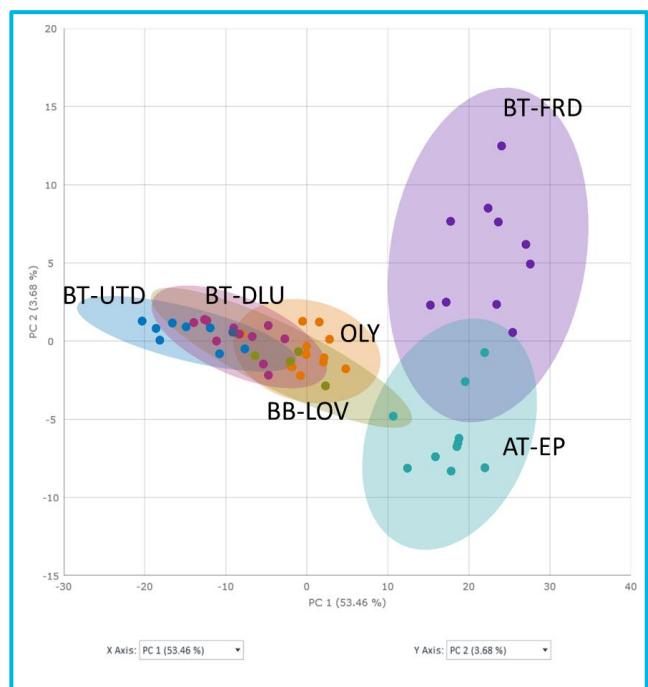
Statistical Treatment of Data

- Features were extracted from all samples and LOESS normalization was carried out.
- 5487 compounds across all samples were measured.
- Comparison between upstream and downstream sites revealed 294 compounds that significantly increased ($p < 0.05$) in downstream locations by a factor of 10.



Results and Discussion

The PCA plot shows a clear difference between upstream and downstream sites after normalization.

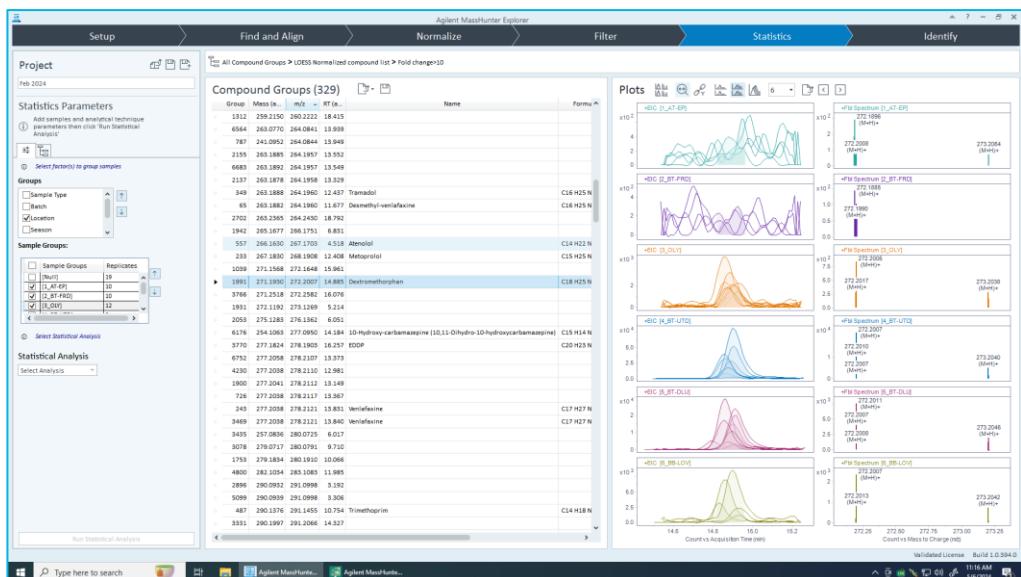


294 compounds were found to be $\times 10$ more significant in downstream sites.

All Compound Groups > LOESS Normalized compound list > Fold change>10									
Group	Mass ...	m/z ...	RT...	Name	Formula	FC (Do...	Log2...	Up/Do...	Abund...
94	241.1236	242.1309	14.020	Erythrohydrobupropion (threo-Dihydrobupropion)	C13 H21 N O	20.77	4.38	up	554220
3834	241.1222	242.1316	13.876	Erythrohydrobupropion (threo-Dihydrobupropion)	C13 H21 N O	11.69	3.55	up	88842
1829	243.1520	244.1695	12.045	Desmethyl-dextrophan	C16 H21 N O	108.07	6.76	up	53232
3870	244.1670	245.1749	16.998			238.71	7.90	up	10532
3280	223.0637	246.0521	18.520			936.25	9.87	up	3947
2565	245.1776	246.1854	11.672			2546.10	11.31	up	68684
4810	246.1724	247.1805	13.860			44.78	5.48	up	9373
5478	249.0583	250.0646	11.081			20.17	4.33	up	14044
341	249.1723	250.1803	10.166	O-Desmethyl-tramadol	C15 H23 N O2	23.82	4.57	up	98645
4837	251.1845	254.1723	14.858			13.41	3.74	up	8113
2216	254.0878	255.0950	9.343			204.08	7.67	up	37544
127	255.0079	256.0153	12.448	Lamotrigine	C9 H7 Cl2 N5	24725.30	14.59	up	690229
4931	259.0174	256.0259	12.441			701.08	9.45	up	27457
357	254.1159	256.1098	12.481			128.84	7.01	up	112408
909	255.1029	256.1098	12.677	Hydroxy-bupropion	C13 H18 Cl N O2	2518.69	11.30	up	160045
5722	255.1023	256.1101	12.426			101.24	6.66	up	21896
4727	255.1946	256.2019	3.596			10.21	3.35	up	10202
644	257.1185	258.1253	12.965			24.89	4.64	up	150449
306	257.1775	258.1852	12.156	Dextrophan	C17 H23 N O	2860.81	11.21	up	145826
2422	259.1569	260.1642	14.745	Propranolol	C16 H21 N O2	13.91	3.80	up	43057
5685	259.1930	260.2013	13.830			495.38	8.95	up	21112
1312	259.150	260.2222	18.415			10.87	3.44	up	25552
6564	263.0770	264.0841	13.939			234.58	7.87	up	56499
787	241.0952	264.084	13.949			76.29	6.25	up	116340
2155	263.1885	264.1957	13.552			11.61	3.54	up	24449
6683	263.1892	264.1957	13.549			16.22	4.02	up	25346
2137	263.1878	264.1958	13.329			54.56	5.77	up	16325
349	263.1888	264.1960	12.437	Tramadol	C16 H25 N O2	19.52	4.29	up	124263
65	263.1882	264.1960	11.677	Desmethyl-venlafaxine	C16 H25 N O2	58.17	5.86	up	379748
2702	263.3265	264.2436	18.792			15.84	3.99	up	16902
1942	265.1677	266.1751	6.831			38.94	5.28	up	18067
557	266.1630	267.1703	4.518	Atenolol	C14 H22 N O3	14.53	3.86	up	145699
233	267.1830	268.1908	12.408	Metoprolol	C15 H25 N O3	28.80	4.85	up	258444

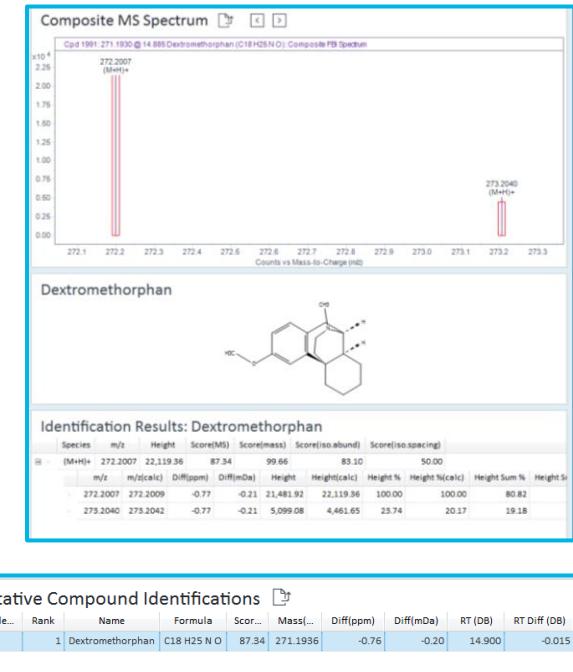
Compound Identification with Home-made databases & Standards

- 27 compounds were identified and confirmed with retention time and MS-MS library



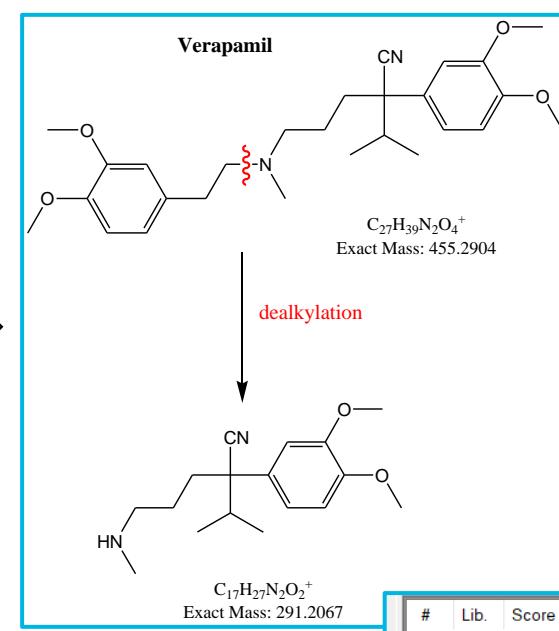
Example:

Dextromethorphan identification. Upstream samples not showing the compound, whereas downstream shows first a spike, then a decrease in area counts (concentration) as it progresses downstream.



Compound Identification with LC-MS NIST Library (2023)

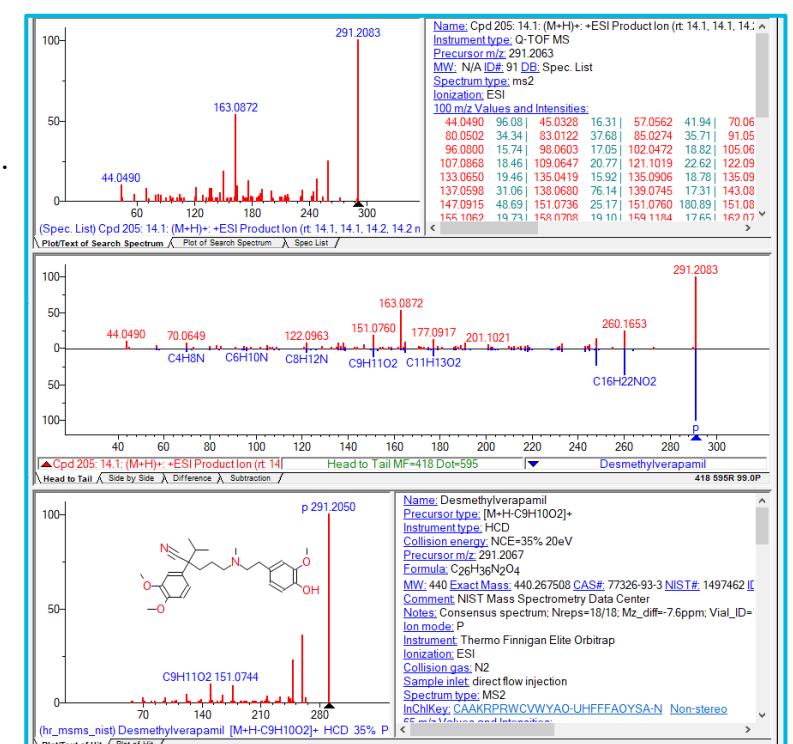
- 247 compounds had available MS-MS data.
- 22 additional compounds were identified and confirmed with the NIST library.



#	Lib.	Score	DotProd	Rev-Dot	Prob. (%)	PSS-Dot	DBs	Prec. Type	Name
1	hr	418	595	847	99.0	629	3 EM	[M+H-C9H10O2]+	Desmethylverapamil [M+H-C9H10O2]+ HCD 35% P=291.2
2	hr	4	77	250	0.99	294	1 M	[M+H-3H2O]+=>[M+H-...	6-[(2,5,5,8a-Tetramethyl-1,4a,6,7,8-hexahydronaphthalen-1

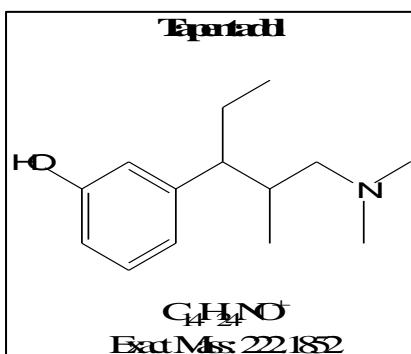
Example:

A verapamil (high blood pressure drug) metabolite was identified by common fragment ions with one of the spectra included in the NIST library.



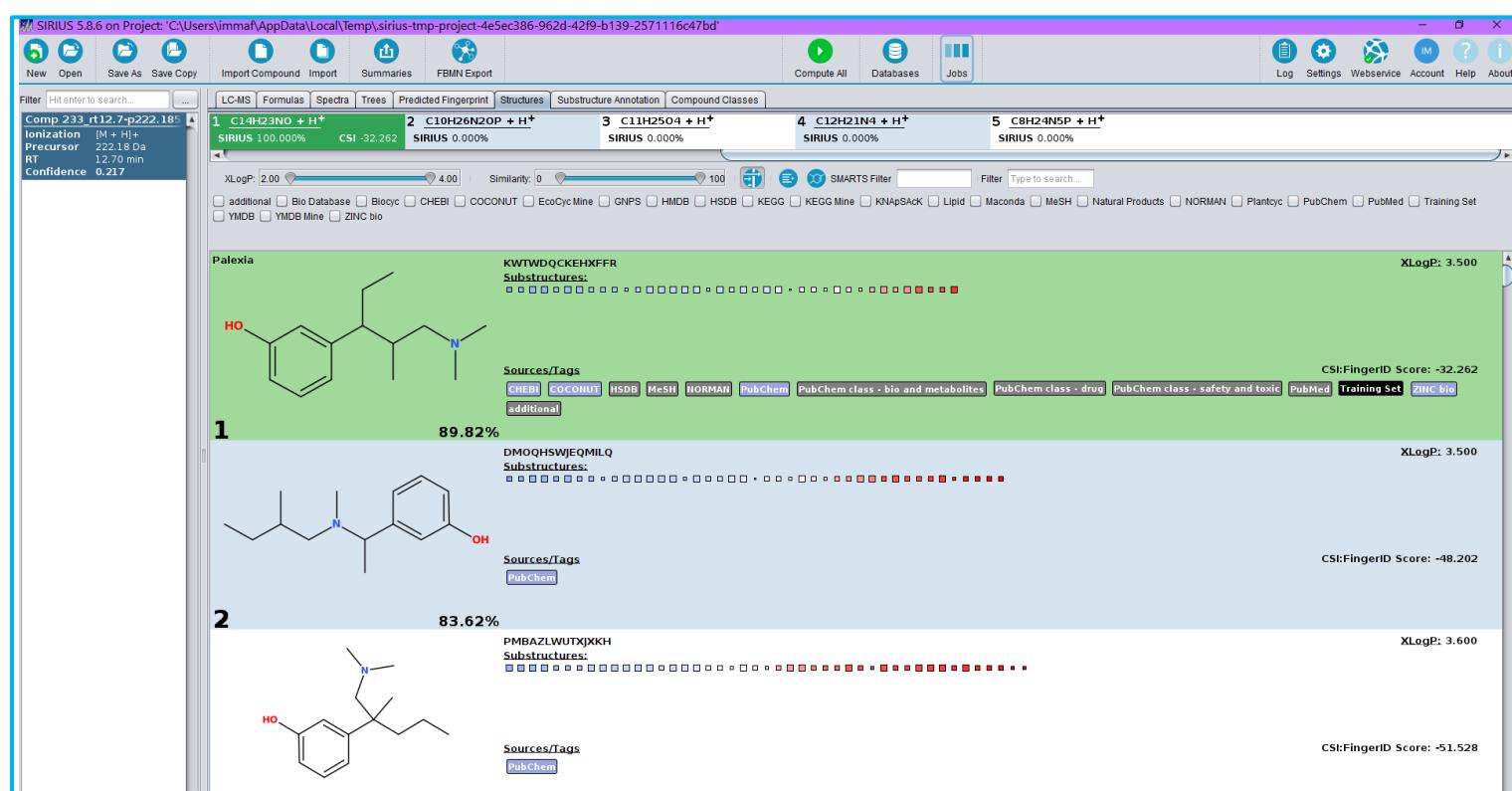
Compound Identification with SIRIUS and CSI:FingerID

- SIRIUS and CSI:FingerID matched and corroborated most of the structures found by NIST.
- This software also generated molecular formulas for those compounds that did not have an MS-MS spectrum available.



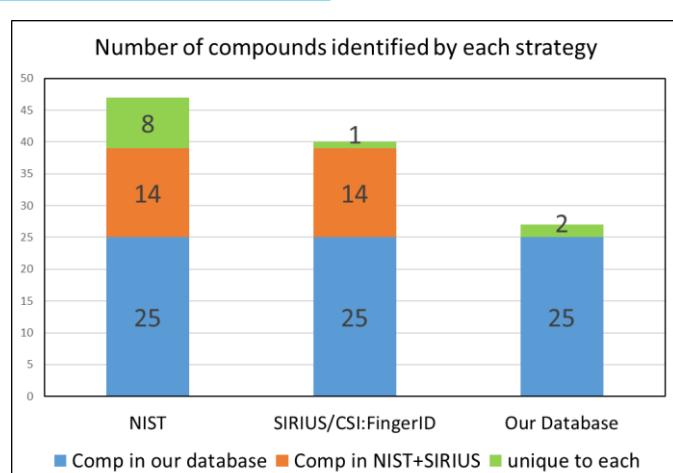
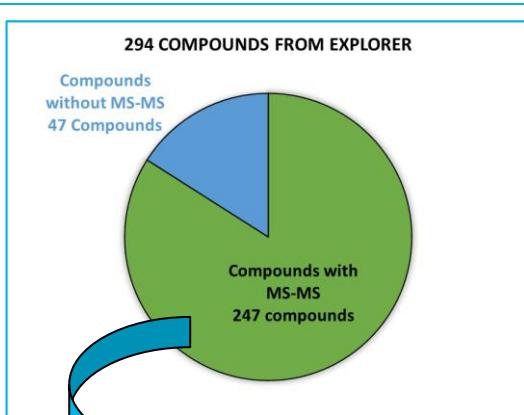
Example:

Opioid drug used to treat pain was identified by SIRIUS



Compounds were placed in 3 different identification categories as a function of several parameters of confidence.

	100% confidence	> 95% confidence	< 95 % confidence
Standard available	✓	✗	✗
Adducts and isotopic pattern present	✓	✓	✓
All fragment ions match chemical structure	✓	✓	✗
Retention time compatible with chemical structure	✓	✓	✗
Compatible in sample type	✓	✓	✗



Conclusions

- Approximately, 20% of 247 compounds containing MS-MS data were identified at >95% confidence by a combination of all three strategies (ChemVista databases, NIST and SIRIUS-CSI:FingerID).
- 75% of the compounds could not be identified with high confidence, but chemical structures were postulated by SIRIUS. An additional manual study of the individual fragment ions for each of these compounds is needed for verification.
- Molecular formulas were obtained for the rest of the compounds (5%), which is insufficient for structural identification.

References

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