Poster Reprint

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

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Introduction

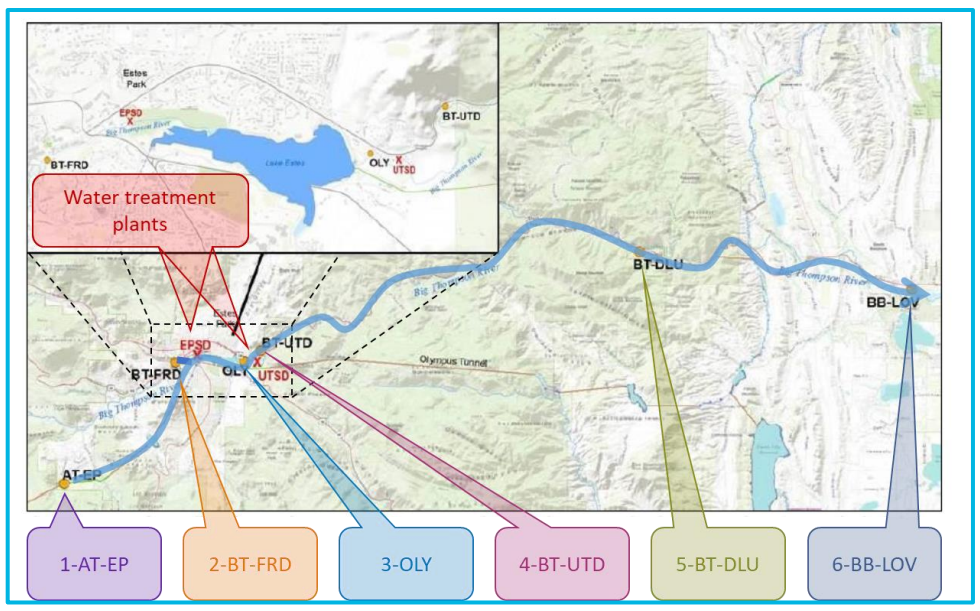
Surface waters are environmentally impacted by wastewater sources. In this work, pristine upstream water sources were compared to downstream locations to find anthropogenic compounds.

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 µL on LC/Q-TOF MS.

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

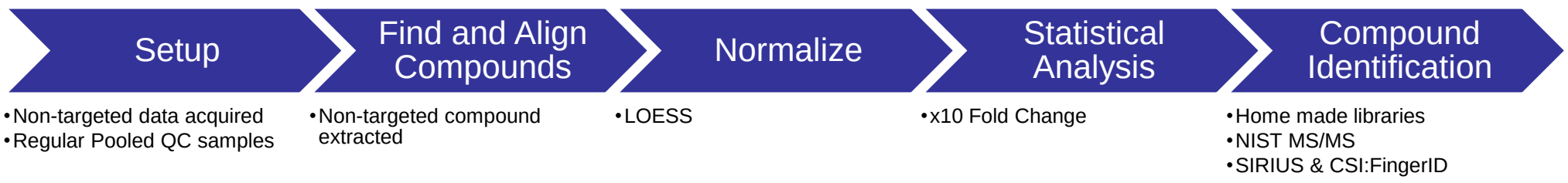
Agilent 1290 Infinity II LC coupled to a 6546 LC/Q-TOF MS

Instrument conditions

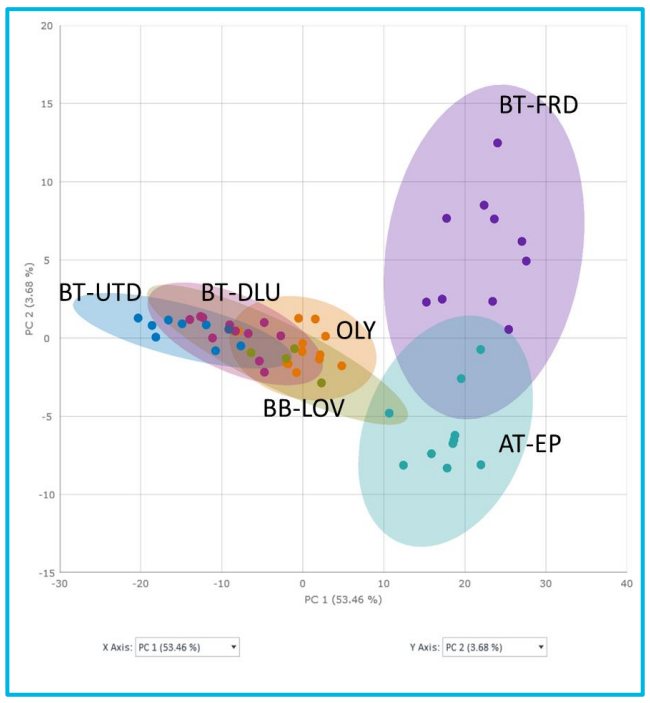
- Reverse phase chromatography (C8 column).
- Data independent (All Ions MS/MS) and data dependent Iterative MS/MS acquisition
- Positive ion electrospray.

Statistical Treatment of Data

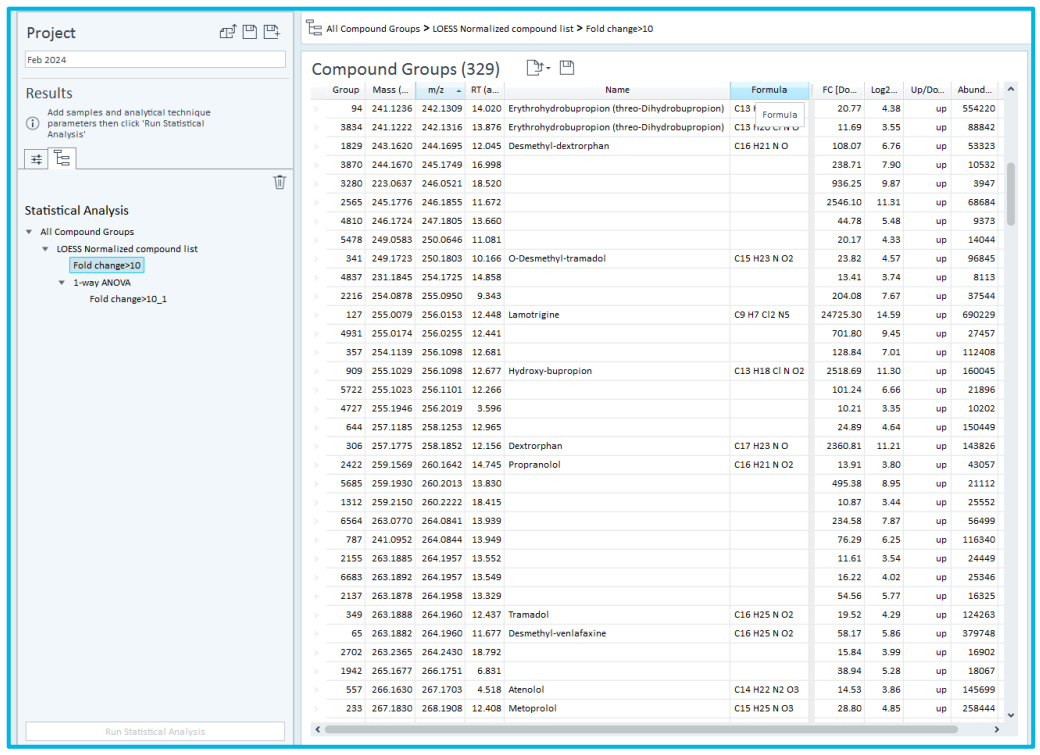
- Features were extracted from all samples and LOESS normalization was carried out.
- 5487 compounds across all samples were measured.
- Comparison between upstream and downstream sites revealed 294 compounds that significantly increased ($p<0.05$) in downstream locations by a factor of 10.



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

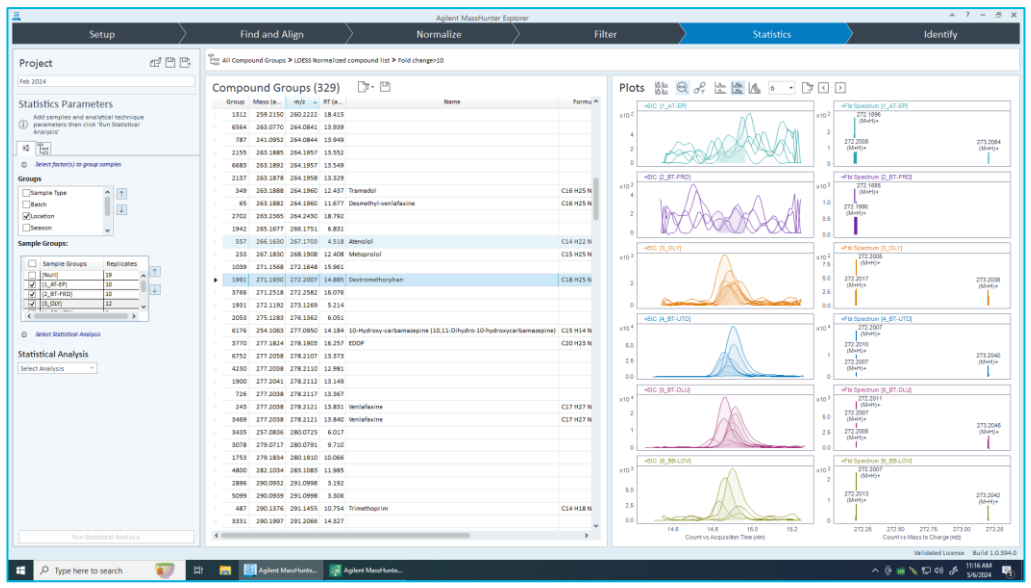


294 compounds were found to be x10 more significant in downstream sites.



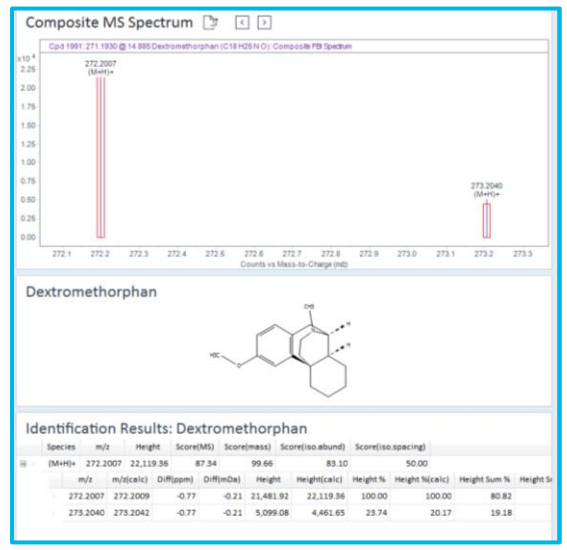
Compound Identification with Home-made databases & Standards

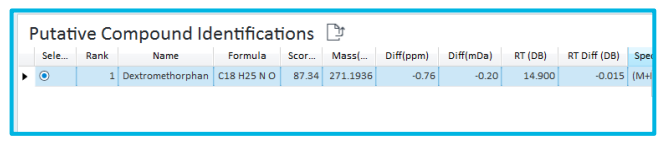
- 27 compounds were identified and confirmed with retention time and MS-MS library



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.



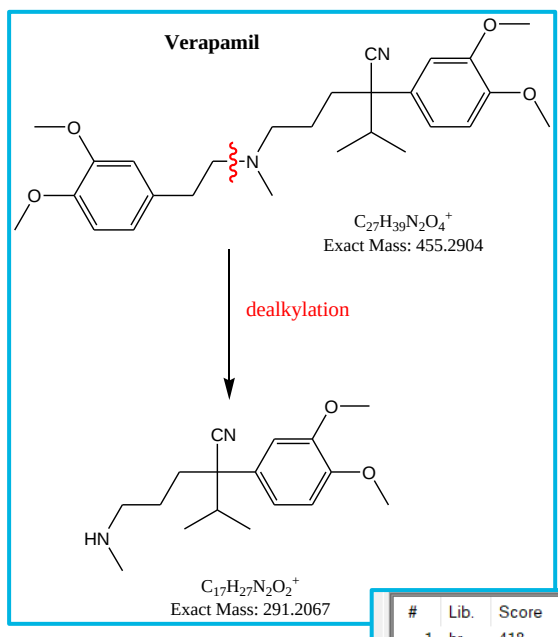


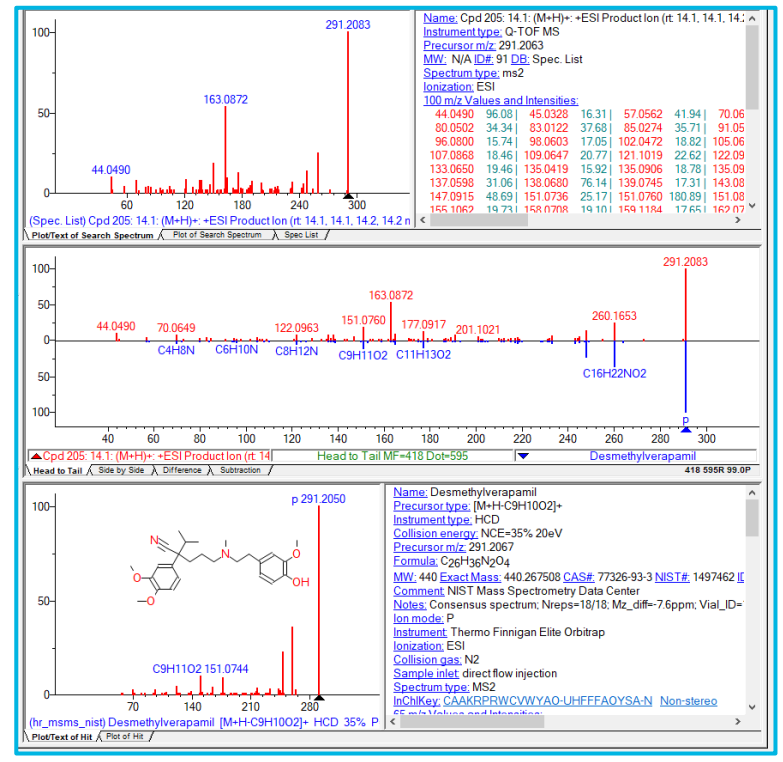
Compound Identification with LC-MS NIST Library (2023)

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

Example:

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



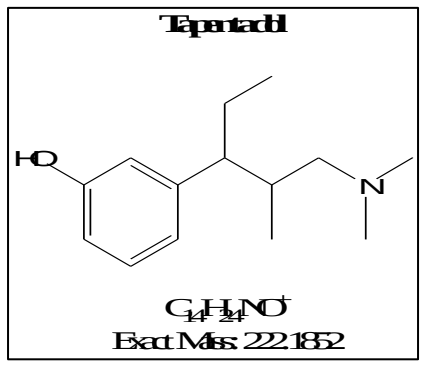


#	Lib.	Score	DotProd	Rev-Dot	Prob. (%)	PSS-Dot	DBs	Prec. Type	Name
1	hr	418	595	847	99.0	629	3 EM	[M+H-C9H10O2]+	Desmethylverapamil [M+H-C9H10O2]+ HCD 35% P=291.2
2	hr	4	77	250	0.99	294	1 M	[M+H-3H2O]+=>[M+H-...	6-[(2,5,5,8a-Tetramethyl-1,4,4a,6,7,8-hexahydronaphthalen-1

Results and Discussion

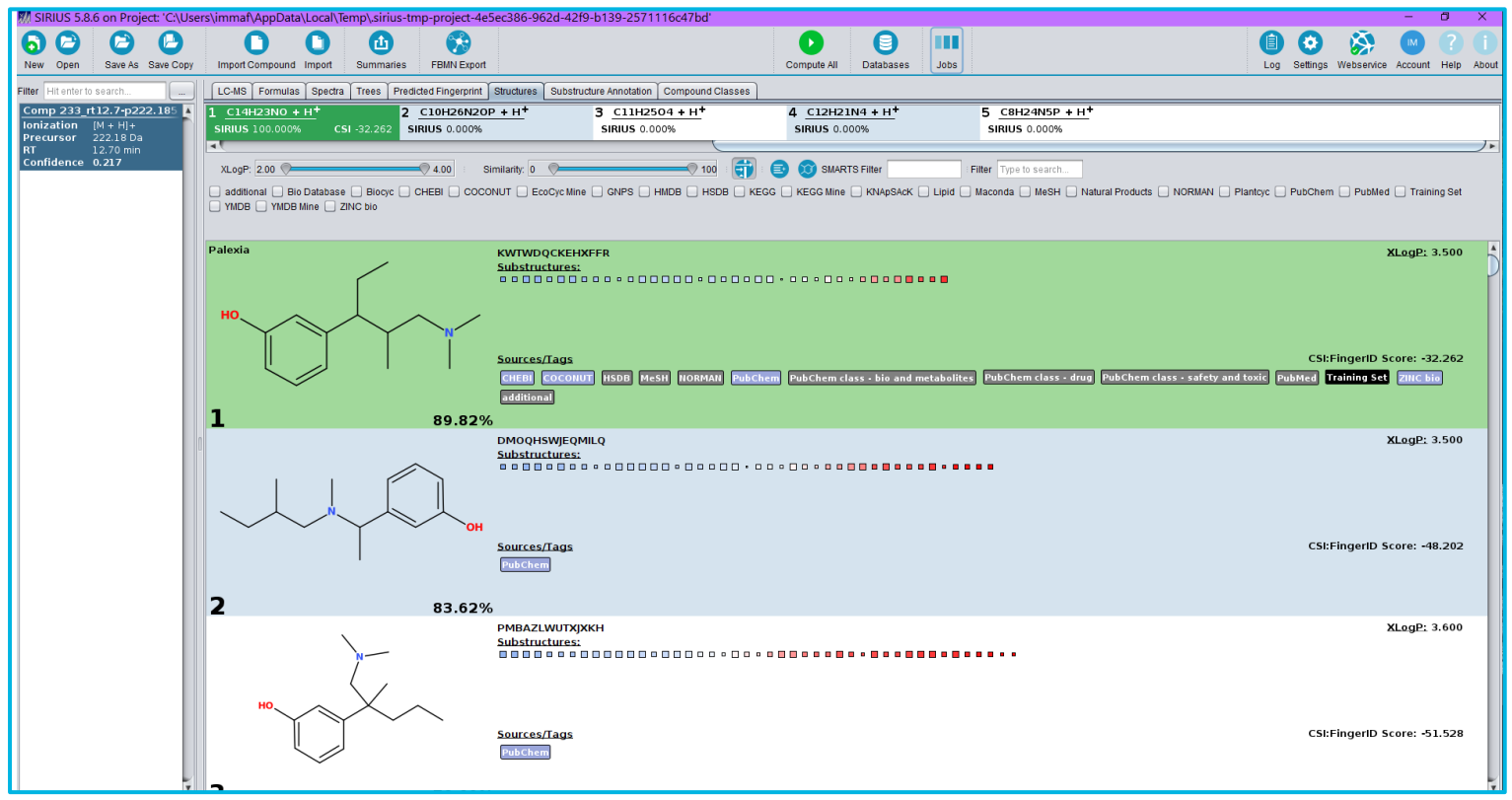
Compound Identification with SIRIUS and CSI:FingerID

- 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.



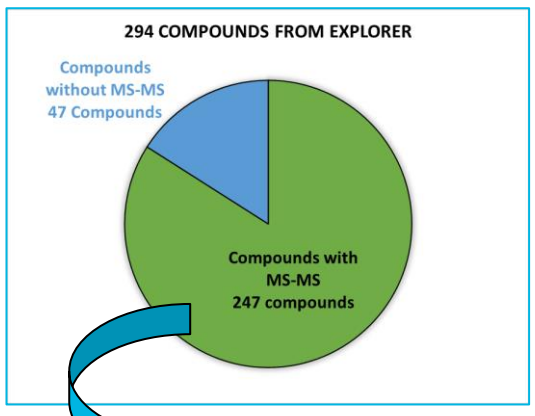
Example:

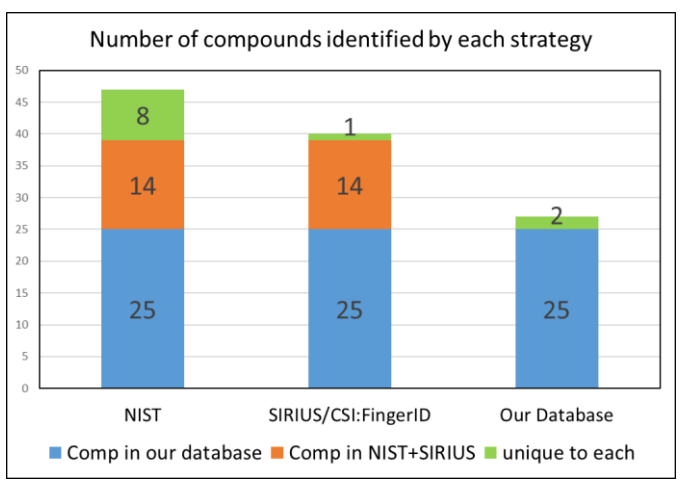
Opioid drug used to treat pain was identified by SIRIUS



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

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





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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

Kai Dührkop, Markus Fleischauer, Marcus Ludwig, Alexander A. Aksenov, Alexey V. Melnik, Marvin Meusel, Pieter C. Dorrestein, Juho Rousu and Sebastian Böcker
SIRIUS4: a rapid tool for turning tandem mass spectra into metabolite structure information, *Nat Methods, 16, 2019.*

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Searching molecular structure databases with tandem mass spectra using CSI:FingerID, *Proc Natl Acad Sci U S A, 112, 2015.*

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