Confident identification of unknown NPS by a unique software workflow: Case study for seized powder

Abstract

The growing importance of identifying drugs of abuse and new psychoactive substances (NPS) requires advanced analytical techniques. High-resolution tandem mass spectrometry (HR-MS/MS) and innovative data processing offer detailed insights into known drug targets and the detection of unexpected compounds. This study employs MetaboScape, a specialized software, for non-target processing of HR-MS/MS data, facilitating the identification of NPS in seized powders. MetaboScape's optimized workflow covers elemental formula calculation and structural confirmation, ensuring robust compound identification. The study showcases MetaboScape's effectiveness in identifying Modafiendz, a fluorinated analog of the DEA Schedule IV* controlled substance modafinil, in a seized powder.

Keywords:
QTOF, Elute UHPLC, MetaboScape, Unknown ID, NPS

Introduction

Identifying drugs of abuse and NPS is gaining greater significance in terms of public health and safety due to several reasons. The use of these substances can lead to serious health risks, including addiction, overdose, mental health issues, and physical harm. Constant innovation in the creation of NPS means that new, potentially harmful substances are frequently entering the market, making it crucial to stay ahead in identification. Many NPS are not yet regulated or controlled by authorities, making them easily accessible, but their safety is often unverified. High resolution tandem mass spectrometry (HR-MS/MS) measurements and advancements in data processing can provide detailed information for known drug targets in a sample and provide mechanisms to identify unexpected compounds.
A major component of the proposed workflow for NPS identification in seized powders is MetaboScape® - a software package designed for non-target processing of HR-MS/MS data. MetaboScape includes a complete set of tools for the identification of unknowns. Each step from the determination of the elemental formula to structural confirmation is supported by an optimized workflow designed to generate full confidence in compound identification. In this study MetaboScape is used to identify Modafiendz and a related oxidation product in a seized powder. Modafiendz is a fluorinated analog of modafinil which is a DEA Schedule IV controlled substance intended to treat sleep and alertness disorders.

Silke Bodendiek[1],
Andrea Kiehne[1]
[1]Bruker Daltonics GmbH & Co. KG, Bremen, Germany

*Drug Enforcement Administration (DEA) US; Schedule IV are drugs, substances, or chemicals that are defined as drugs with a low potential for abuse and low risk of dependence; an example of a Schedule IV substance is: diazepam (Valium®).

MetaboScape - The path from unknowns to identification

The MetaboScape based workflow is a powerful tool for the characterization of unknowns. The full integration of SmartFormula, CompoundCrawler, and MetFrag [1] enables reliable molecular formula determination, structure assignment based on public database searching, and verification through comprehensive *in-silico* fragmentation for the confident identification of new substances (Figure. 1).

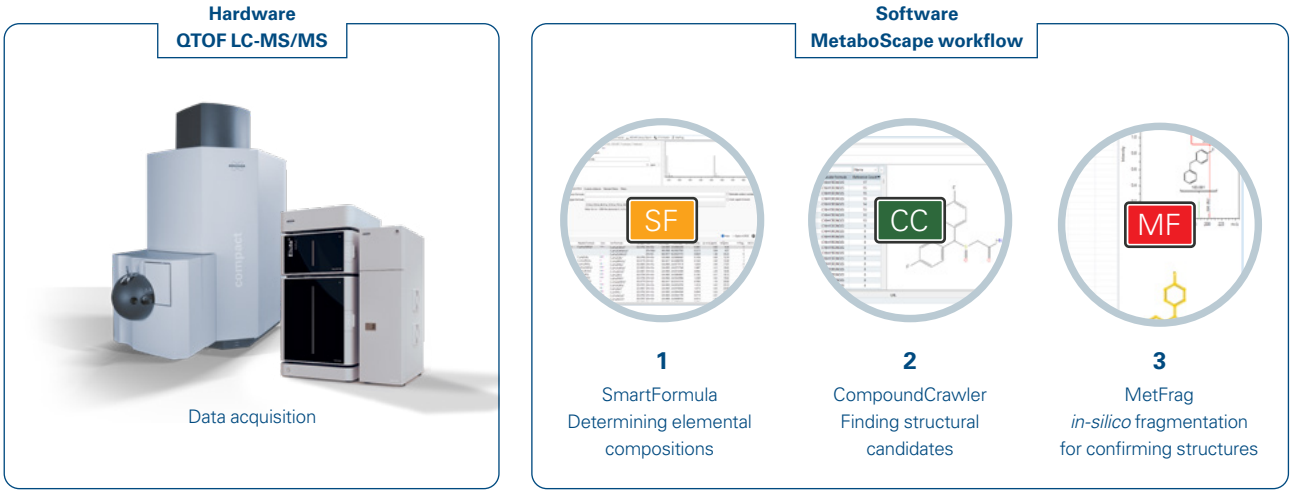


Figure 1. Analysis workflow including QTOF LC-MS/MS data acquisition and MetaboScape data processing to identify unknowns, supported by SmartFormula, CompoundCrawler and MetFrag tools.

When routine screening fails

A major challenge for routine screening is the constant appearance of new substances in the drug of abuse market. As a result, these new major components may not be identified in routine testing.

A seized powder sample serves as an example. The MS Base Peak Chromatogram (BPC) in Figure 2 shows two major unidentified peaks at 7.23 min and 7.66 min, in addition to some identified low-concentration compounds.

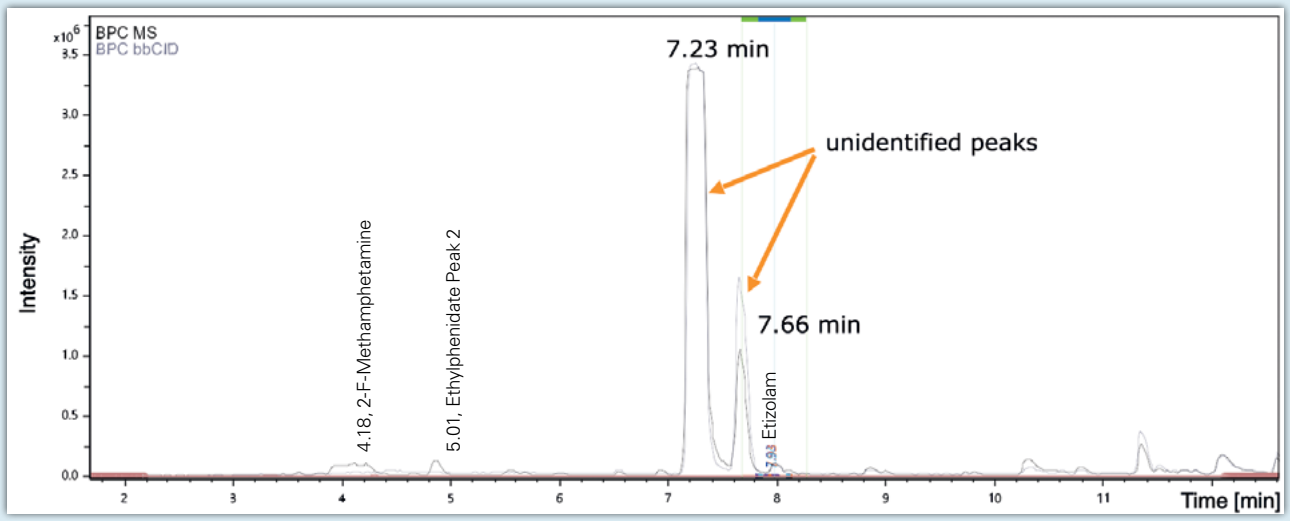


Figure 2. Base Peak Chromatogram (BPC) of a seized powder with unidentified compounds at 7.23 and 7.66 min.

MS and MS/MS spectra of the major peak at 7.23 min are shown in Figure 3. The signal at *m/z* 324.0868 represents the $[M+H]^+$ ion, and the other MS signals at this retention time are cationized species of the same compound. The signal at *m/z* 203.0678 is possibly an in-source CID fragment of the main compound.

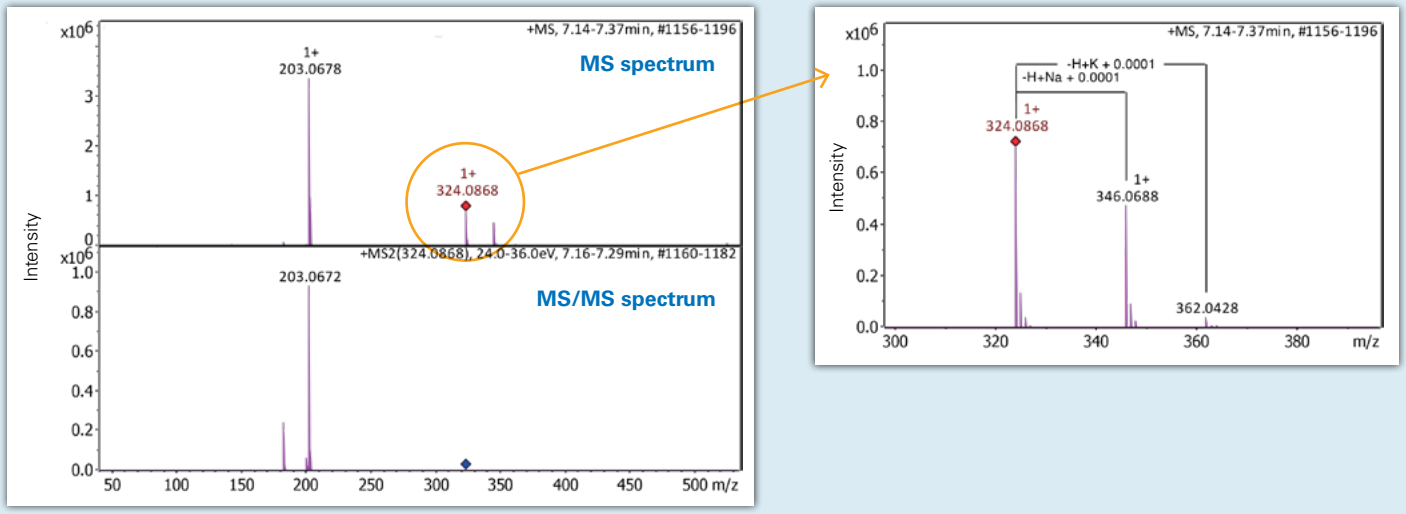


Figure 3. MS and MS/MS spectra of *m/z* 324.0868 at 7.23 min.

3-Step Workflow: MetaboScape identifies the unknowns

The 3-step workflow in MetaboScape was applied to identify the unknown major peak at 7.23 min.

SF

Step 1: Determining the elemental composition

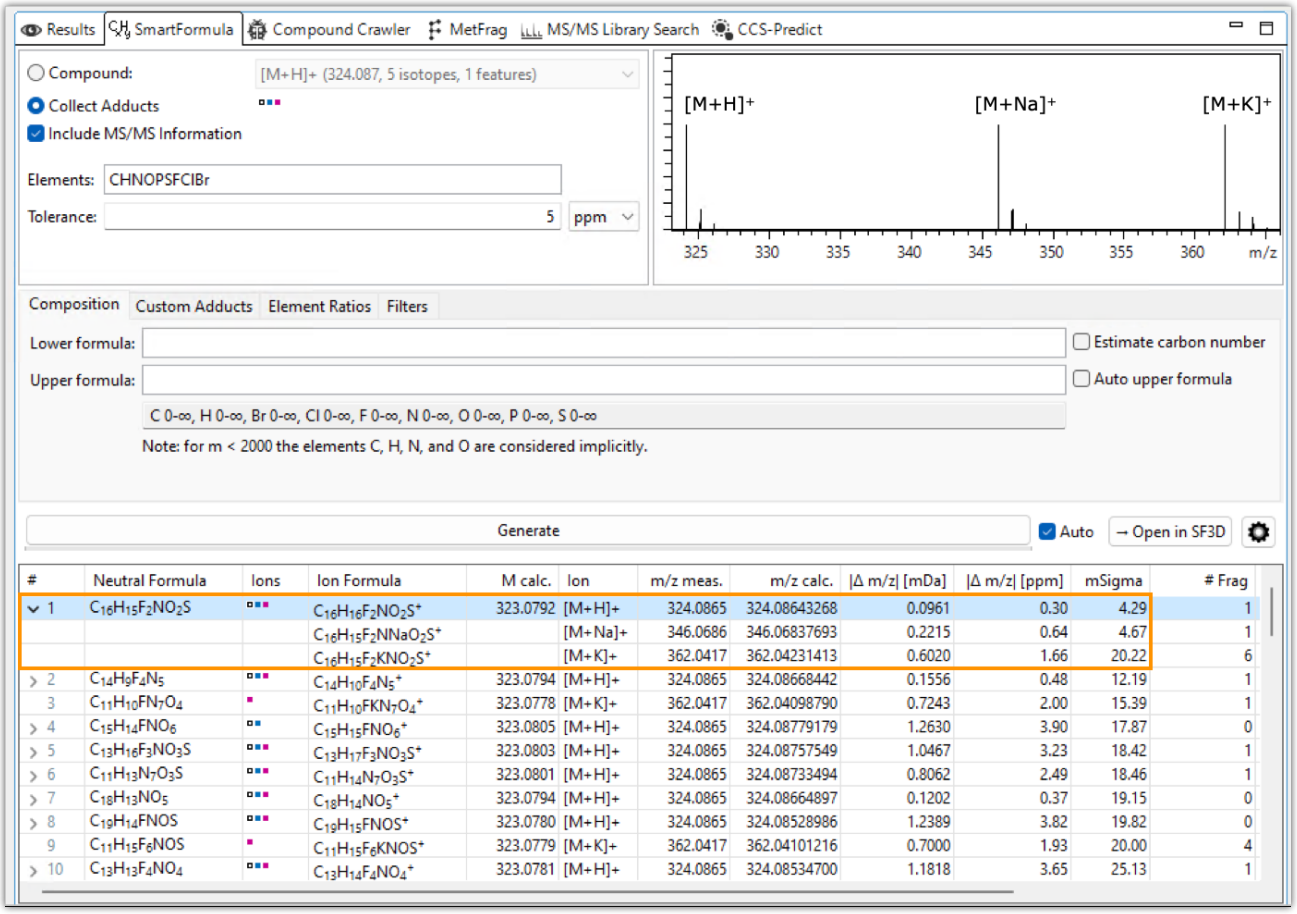
SmartFormula was used to calculate the elemental composition of the unknown substance at *m/z* 324.0868. By default, MetaboScape considers the elements C, H, N, O, P, and S. Furthermore, F, Cl, and Br were added as these elements are common in NPS.

The results are filtered by even electron configurations and according to the "Seven Golden Rules" by Fiehn and Kind [2] (Figure 4).

The scoring of the results is based on two criteria:

1. Mass accuracy of the precursor ion in ppm or mDa
2. Agreement of the isotope pattern expressed as mSigma value

The hit #1 $C_{16}H_{15}F_2NO_2S$ provides the best isotope pattern fit and an excellent mass accuracy for the $[M+H]^+$ as well as for the sodium and potassium adducts.



#	Neutral Formula	Ions	Ion Formula	M calc.	Ion	m/z meas.	m/z calc.	|Δ m/z| [mDa]	|Δ m/z| [ppm]	mSigma	# Frag
1	$C_{16}H_{15}F_2NO_2S$		$C_{16}H_{16}F_2NO_2S^+$	323.0792	[M+H]+	324.0865	324.08643268	0.0961	0.30	4.29	1
			$C_{16}H_{15}F_2NNaO_2S^+$		[M+Na]+	346.0686	346.06837693	0.2215	0.64	4.67	1
			$C_{16}H_{15}F_2KNO_2S^+$		[M+K]+	362.0417	362.04231413	0.6020	1.66	20.22	6
2	$C_{14}H_9F_4N_5$		$C_{14}H_{10}F_4N_5^+$	323.0794	[M+H]+	324.0865	324.08668442	0.1556	0.48	12.19	1
3	$C_{11}H_{10}FN_7O_4$		$C_{11}H_{10}FKN_7O_4^+$	323.0778	[M+K]+	362.0417	362.04098790	0.7243	2.00	15.39	1
4	$C_{15}H_{14}FNO_6$		$C_{15}H_{15}FNO_6^+$	323.0805	[M+H]+	324.0865	324.08779179	1.2630	3.90	17.87	0
5	$C_{13}H_{16}F_3NO_3S$		$C_{13}H_{17}F_3NO_3S^+$	323.0803	[M+H]+	324.0865	324.08757549	1.0467	3.23	18.42	1
6	$C_{11}H_{13}N_7O_3S$		$C_{11}H_{14}N_7O_3S^+$	323.0801	[M+H]+	324.0865	324.08733494	0.8062	2.49	18.46	1
7	$C_{18}H_{13}NO_5$		$C_{18}H_{14}NO_5^+$	323.0794	[M+H]+	324.0865	324.08664897	0.1202	0.37	19.15	0
8	$C_{19}H_{14}FNOS$		$C_{19}H_{15}FNOS^+$	323.0780	[M+H]+	324.0865	324.08528986	1.2389	3.82	19.82	0
9	$C_{11}H_{15}F_6NOS$		$C_{11}H_{15}F_6KNOS^+$	323.0779	[M+K]+	362.0417	362.04101216	0.7000	1.93	20.00	4
10	$C_{13}H_{13}F_4NO_4$		$C_{13}H_{14}F_4NO_4^+$	323.0781	[M+H]+	324.0865	324.08534700	1.1818	3.65	25.13	1

Figure 4. Proposals for the elemental composition of the unknown peak at *m/z* 324.0865.
The first hit shows the best combination of mass error and isotope pattern fit.

Step 2: Finding structural candidates

To identify possible structures for the elemental composition $C_{16}H_{15}F_2NO_2S$, public databases such as Chemspider, PubChem and ChEBI are searched with CompoundCrawler.

The search yielded more than 1000 potential structural candidates, 40 of which are listed in Figure 5

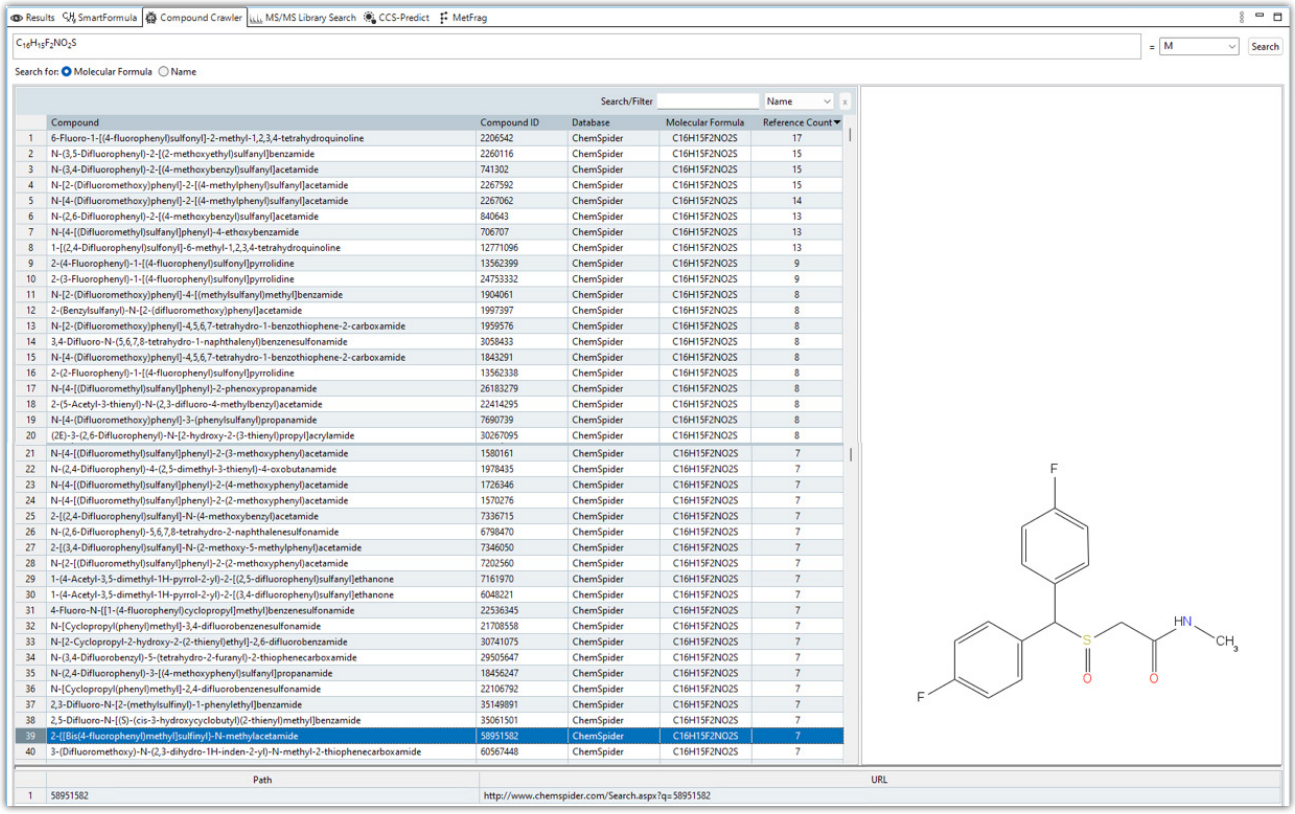


Figure 5. CompoundCrawler found more than 1000 potential structural candidates in the public databases of Chemspider, PubChem and ChEBI which need to be further evaluated.

MF

Step 3: Confirming structures

The first 40 structure candidates of the list in CompoundCrawler were subjected to *in-silico* fragmentation by MetFrag [1], and the calculated *in-silico* fragments were matched with the experimental MS/MS peak list. The top hit 2-{[bis(4-fluorophenyl)methyl] sulfinyl}-N-methylacetamide, a new psychoactive substance also known as Modafiendz, shows by far the best agreement with the experimental data, resulting in the maximum score of 1 (Figure 6). The score of the second hit is significantly lower at 0.686.

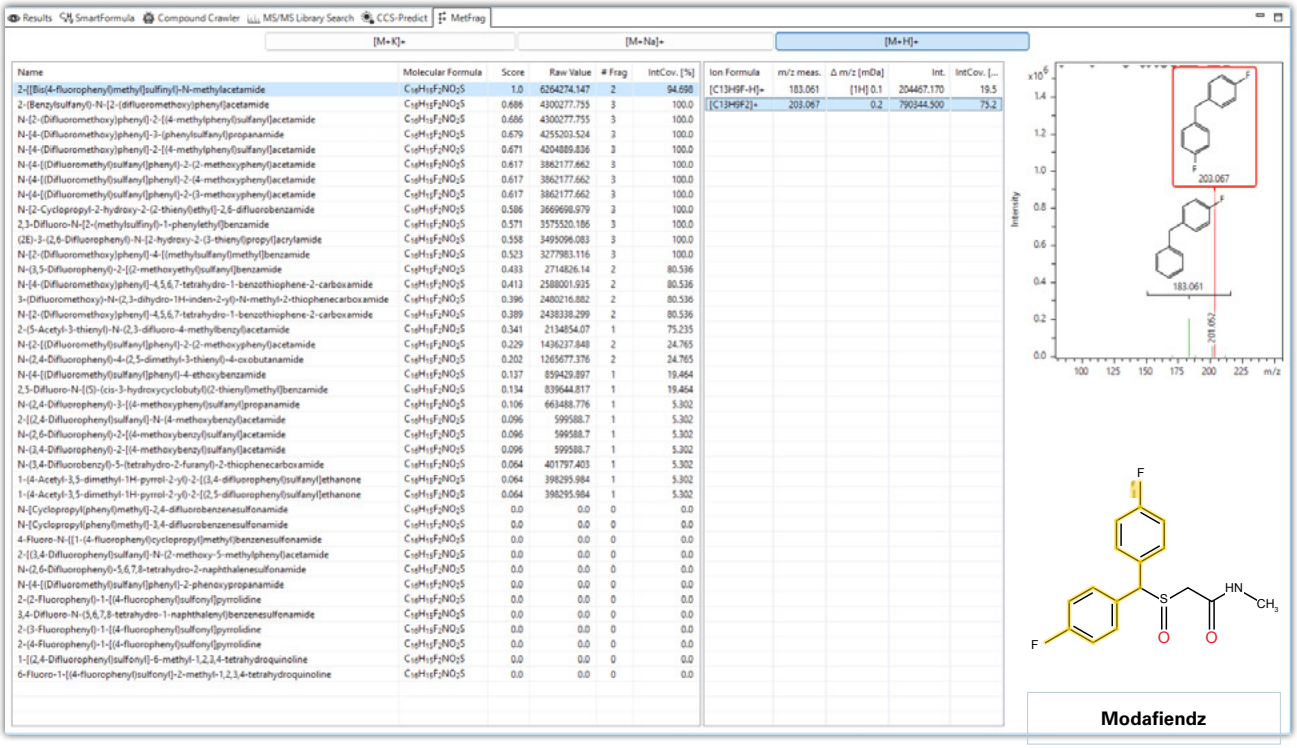
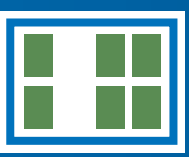


Figure 6. Matching the theoretical fragments of the potential structures with the experimental MS/MS spectrum. Modafiendz, the top hit with the maximum score of 1, shows by far the best agreement with the experimental data, compared to the second rank with a score of 0.686.

Annotation Quality (AQ) scoring

1 Mass accuracy
2 RT deviation
3 mSigma value
4 MS/MS score

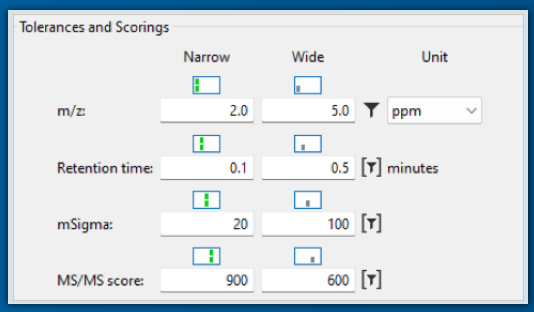
Annotation Quality (AQ) scoring: Each bar represents a different quality attribute.



high confidence

not applicable for unknowns

Tolerances and scoring limits for the annotation can be customized according to individual needs and conditions.
The settings for the seized powder analysis were:



Intuitive Annotation Quality score for easy result assessment

The quality of the final identification result is illustrated by the color-coded Annotation Quality (AQ) score. Green squares indicate excellent mass accuracy, retention time agreement, isotope pattern fit and MS/MS spectra match. This is particularly useful for visualizing results for entire LC-MS/MS runs.

Annotated feature after calculation of the molecular formula: Perfect results in mass accuracy and isotope pattern matching.

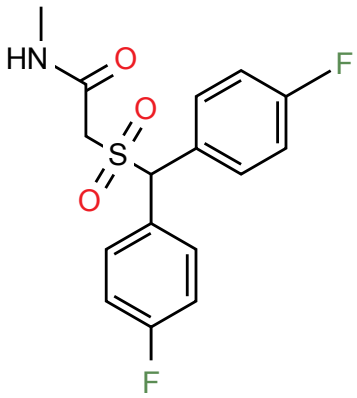
RT [min]	m/z meas.	M meas.	Ions	Δm/z [ppm]	mSigma	Molecular Formula	Annotations	AQ	MS/MS
7.23	324.08653	323.07897		0.297	4.3	$C_{16}H_{15}F_2NO_2S$	SF		

Final result: Annotated feature after completion of the unknown identification workflow.

RT [min]	m/z meas.	M meas.	Ions	Δm/z [ppm]	mSigma	Molecular Formula	Name	Annotations	AQ	MS/MS
7.23	324.08653	323.07897		0.297	4.3	$C_{16}H_{15}F_2NO_2S$	2-{[Bis(4-fluorophenyl)methyl]sulfinyl}-N-methylacetamide	MFSFCC	M	

Identification of the oxidation product of Modafiendz

Using the same workflow, the second major peak at 7.66 min with m/z 340.0818 was identified as 2-{[Bis(4-fluorophenyl)methyl]sulfonyl}-N-methylacetamide ($C_{16}H_{15}F_2NO_3S$), which is an oxidation product of Modafiendz.



2-{[Bis(4-fluorophenyl)methyl]sulfonyl}-N-methylacetamide

Molecular Formula	$C_{16}H_{15}F_2NO_3S$
Average mass	339.357 Da
Monoisotopic mass	339.074066 Da
ChemSpider ID	30059527

From http://www.chemspider.com

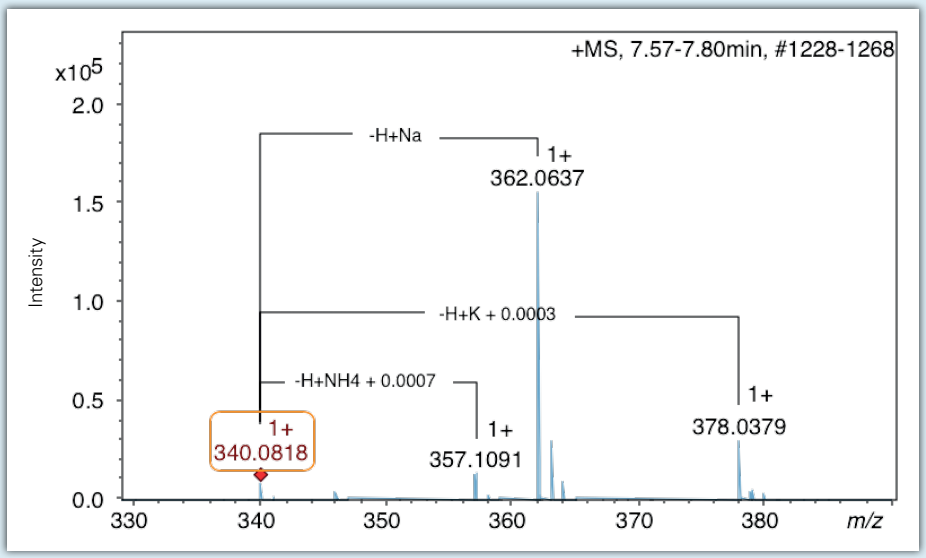


Figure 7. Mass spectrum of the second major peak at 7.66 min and the identified compound information.

The application of the workflow shows the successful identification of both Modafiendz and its oxidation product.

Conclusion

The ability to confirm unknown chemical identities quickly and accurately is critical for law enforcement, security, and public safety. The demonstrated workflow for analyzing seized powders and processing HR-MS/MS data through the software tools available in MetaboScape successfully identified an NPS that was not included in the initial screen and would not have been found in a targeted approach. The tools for identifying unknowns are combined in an intuitive and automated workflow and are fully integrated into the MetaboScape software.

SF
- SmartFormula allows for determination of elemental composition.

CC
- CompoundCrawler enables public structural database search functions.

MF
- MetFrag compares predicted MS/MS fragments to experimental data.

The combined workflow with these three tools reduces the list of candidates subsequently and typically leads to a clear and reliable identification of unknown peaks.

Once identified, the new compound can be included in the standard screening method for future analyses.

References

[1] Wolf S., Schmidt S., Müller-Hannemann M., Neumann S. *In silico* fragmentation for computer assisted identification of metabolite mass spectra. BMC Bioinformatics 2010, 201011:148. doi: 10.1186/1471-2105-11-148

[2] Kind, T., Fiehn, O. Seven Golden Rules for heuristic filtering of molecular formulas obtained by accurate mass spectrometry. BMC Bioinformatics 8, 105 (2007). https://doi.org/10.1186/1471-2105-8-105

Bruker Switzerland AG

Fällanden · Switzerland
Phone +41 44 825 91 11

Bruker Scientific LLC

Billerica, MA · USA
Phone +1 (978) 663-3660

marketing.bams.emea@bruker.com - www.bruker.com