DART-HRMS for the rapid screening of seized drugs: A study on authentic paper samples

Abstract

Increased backlogs of seized drug samples, high turnaround times and the appearance of a high number of new psychoactive substances (NPS) are key challenges within the forensic community worldwide. This application note showcases a comprehensive workflow provided by the Seized Drug Suite featuring the powerful combination of Direct Analysis in Real Time with High Resolution Mass Spectrometry (DART-HRMS). This unmatched pairing provides ultra-fast acquisition speed with high information depth for targeted and non-target analyses.

Keywords:
DART; Seized Drug Suite; seized drugs; spectral library search; unknown ID

Introduction

Forensic labs worldwide are challenged by an ever-increasing number of seized drug samples and a continuous influx of novel psychoactive substances. The NFLIS-Drug 2019 Survey of Crime Laboratory Drug Chemistry Sections Report revealed that laboratories have average turnaround times of 60 days and 1,800 drug cases backlogged [1]. This shows that the established techniques are pushed to their limits regarding the current demand in sample throughput.
A potential alternative is the adoption of Direct Analysis in Real Time in combination with High Resolution Mass Spectrometry (DART-HRMS) for screening analysis. Early adopters have been acknowledging DART-MS already for its ability to generate reliable analytical results much faster and easier than established techniques [2]. However, what has been missing so far is a seamless integration of DART into routine testing workflows.

In this application note, we showcase the usage of DART-HRMS for the investigation of paper samples seized at routine controls in prisons and forensic psychiatry institutions. A frequently used method for smuggling drugs into the prison is based on soaking paper used for letters in a drug mixture, allowing it to dry and then writing on it. After being received by the prisoner and the letter read, they smoke portions of it to consume the drug. Authorities have become aware of this approach and developed methods for screening letters sent into the prison to counter it.

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Fast seized drug with the Seized Drug Suite

Analysis of authentic paper samples

Paper samples seized at routine controls in prisons and forensic psychiatry institutions were screened using the Seized Drug Suite. The Seized Drug Suite is a hardware and software bundle with optimized methods for acquisiton, processing and reporting specifically designed for the analysis of seized drug samples. The samples were extracted with MeOH, and 3 µL of the extract were deposited onto a DART QuickStrip® card.

All analyses were run with a DART JumpShot® source coupled to a QTOF mass spectrometer (both Bruker). Helium was used as ionization gas at a temperature of 275 °C. Data was acquired in positive ionization mode with AutoMS/MS using stepped collision energies of 24 eV and 36 eV.

For subsequent confirmation analysis the TargetScreener HR was used. Therefore, an Elute UHPLC was coupled to the QTOF instrument. Switching sources from DART to Vacuum Insulated Probe-Heated ElectroSpray Ionization (VIP-HESI) source is feasible within a few minutes without breaking the vacuum due to the easy exchange mechanism.

Data acquisition

The fast data acquisition speed of the Bruker QTOF instrument allows to receive full scan MS information as well as MS/MS fragmentation spectra within the very short run time of the DART analysis which is typically 3 s to 60 s. During the acquisition, the QTOF mass spectrometer alternates between MS and data dependent AutoMS/MS with high collision energy. AutoMS/MS parameters were carefully optimized to receive high quality fragment data on a maximum number of selected precursors. One key parameter for the number of precursors is the "active exclusion" which assures fragmentation of low abundant precursors in the presence of high abundant ones.

Spectral library search

The compound identification is supported by spectral library searches. Here, an open library concept is employed, allowing the free choice of libraries* to be integrated into the workflow. The here used Seized Drug Suite comes with a spectral library contaning MS/MS spectra for more than 280 compounds acquired using DART-QTOF-MS.
As there is no significant difference in the fragmentation patterns between DART and ESI, there is no restriction to only use DART-specific libraries. Furthermore, own spectral libraries can be created and modified. Available libraries include among others the NIST DART-MS Forensic Database (2021), the "Maurer, Meyer, Helfer, Weber: LC-HR-MS/MS Library of Drugs, Poisons, and Their Metabolites" and the NIST/EPA/NIH Mass Spectral Library 2023.

Sample report

The sample report is automatically generated and contains all relevant information including a data table with sample and method information, an overview of the library search results and spectral details for each compound identified.

Spectral libraries must be in one of the following data formats to be supported: Library file (.mlb), ASCII Library File (*.library) and NIST ASCII MS Library File (*.msp).

Overview of the sample preparation process, hardware and software used

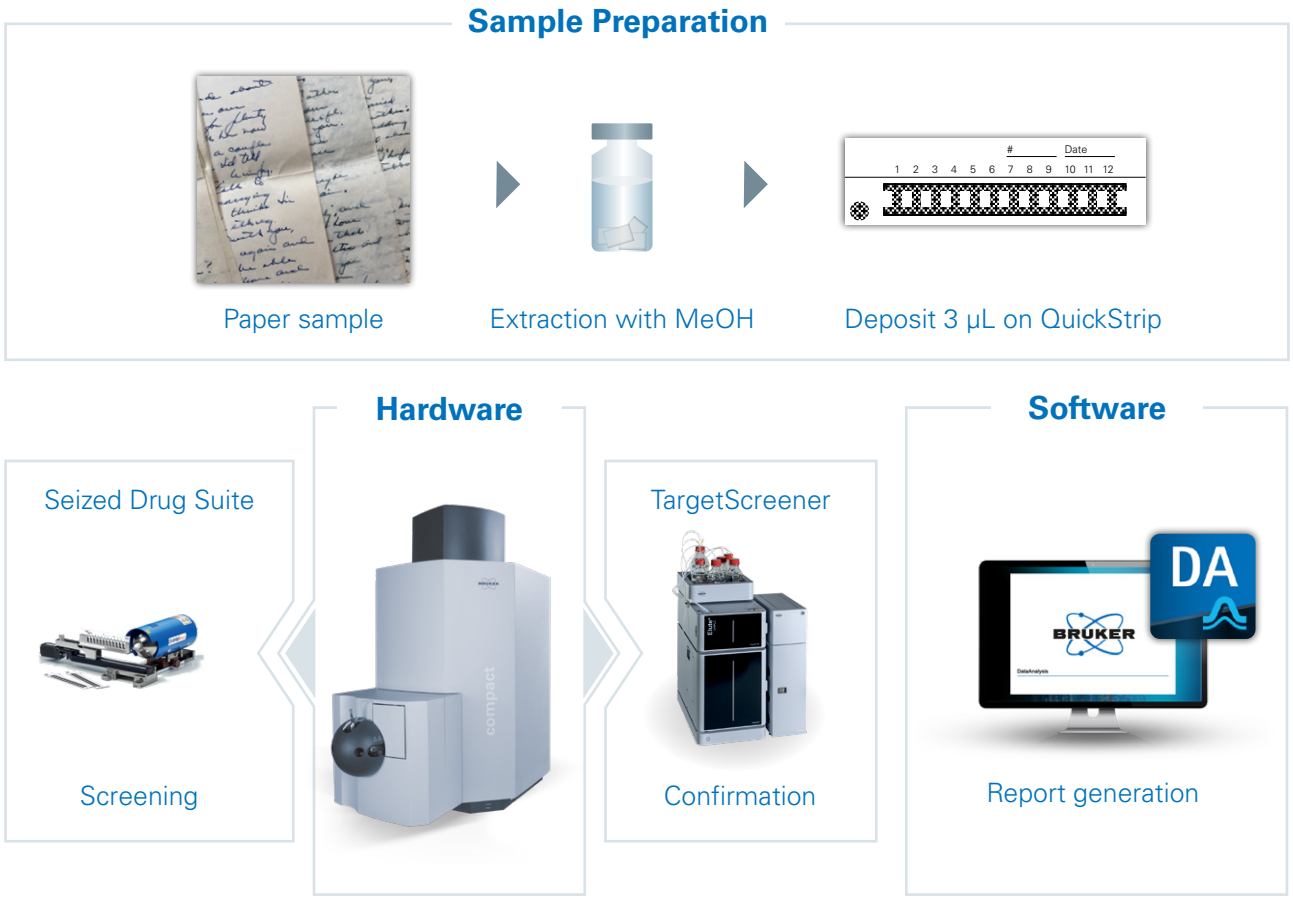
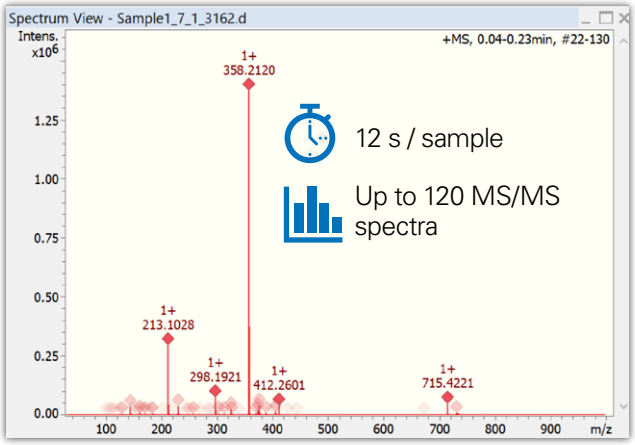


Figure 1. Overview of the sample preparation process, hardware and software applied.

Workflow for screening of seized drug samples

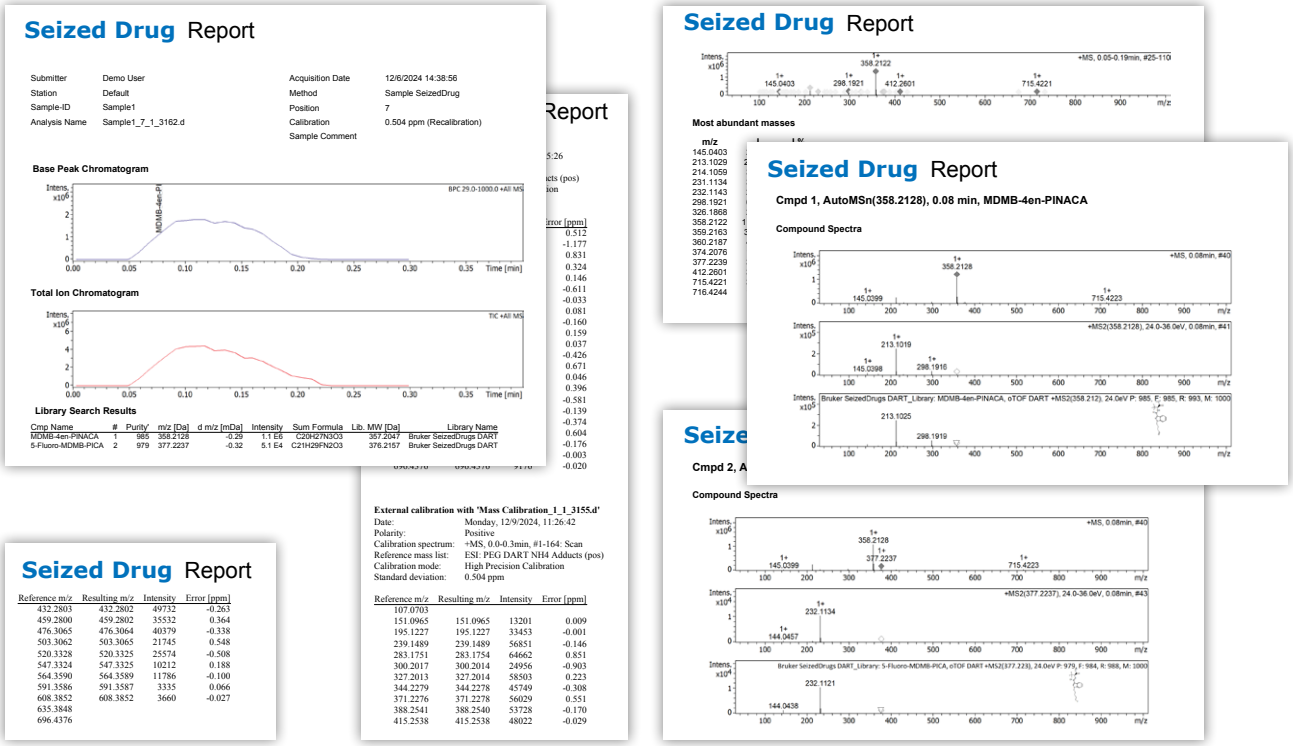
Parallel acquisition of full scan MS and data-dependent MS/MS information. Sample analysis time of 12 s delivers up to 120 MS/MS spectra

DART-HRMS/MS

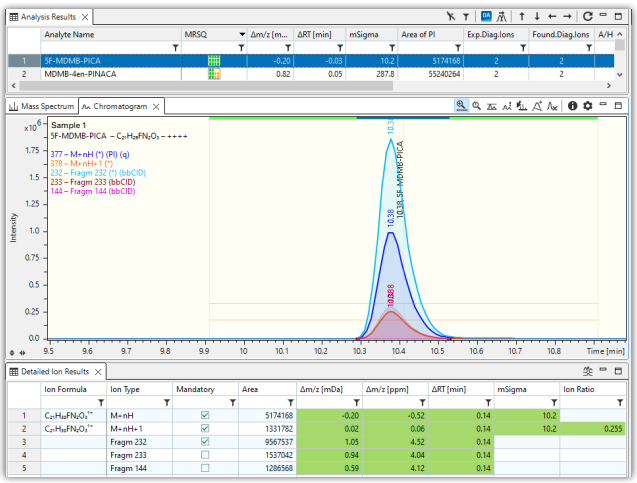


Report is generated automatically when the analysis is finished

Sample Report



LC-QTOF-MS Confirmation



TargetScreener HR method was used for confirmation by RT, *m/z*, sigma and qualifier ions

TargetScreener is a comprehensive screening solution. It includes a high-quality forensic database with over 1,000 compounds of forensic relevance that is updated on a regular basis.

Figure 2. Workflow for screening of seized drug samples using DART-HRMS

Results

Authentic paper samples were analyzed using the Seized Drug Suite workflow. Figure 2 illustrates the results for one selected paper sample. Data acquisition was completed within 12 s with approximately 120 individual acquired MS/MS spectra. Two synthetic cannabinoids, MDMB-4-en-PINACA and 5-Fluoro-MDMB-PICA were successfully identified with library match scores greater than 900. Both were confirmed subsequently through LC-QTOF-MS analysis. Summarizing all analyzed paper samples, synthetic cannabinoids were the dominant drug effect group found. This group comprises substances which are functionally similar to THC (d9-tetrahydrocannabinol) as they also interact with the cannabinoid receptors. In the last years, synthetic cannabinoids have taken the lead among NPS. Between December 2021 and May 2023, 40% of newly reported NPS were synthetic cannabinoids [3]. Additionally, as of 2022, MDMB-4-en-PINACA emerged as the most frequently reported synthetic cannabinoid in the United States, while 5-Fluoro-MDMB-PICA ranked as the third most prevalent [4].

Identification of unknowns

With the constant emergence of these NPS it is quite common that seized drug samples produce signals that don't match any existing library entries but may still be of forensic relevance. The elucidation of the identity of these unknown compounds is another key strength of high-resolution mass spectrometers. Compared to low resolution instruments like quadrupoles, QTOF mass spectrometers measure the mass of an ion with an accuracy that enables the determination of the elemental composition of unknown compounds. Matching the experimental isotope pattern of the found elemental composition with the theoretical one provides a second criterion to validate the formular prediction. Finally, structural information is received by the MS/MS fragmentation spectra which can be compared with *in-silico* fragmentation patterns of the compound structure found in the databases. Hence, the compound identification can be narrowed down step by step based on these three criteria.

Bruker's software MetaboScape® guides the user in a seamless three-step workflow from initial spectral features to the final annotations in a automated and rapid way. The subsequent page illustrates a detailed walkthrough of this process, using the annotation of the synthetic cannabinoid ADB-BUTINACA as an example.

First, the **SmartFormula** tool in MetaboScape generates a list of the best matching elemental compositions based on the determined accurate mass and the fit of isotope patterns. Here, the user can provide input which elements should be considered.

In the next step, the **CompoundCrawler** tool searches in publicly available databases such as PubChem and in a local, customizable database called AnalyteDB for structure candidates corresponding to the elemental compositions. To address the challenge of structures needing to be listed in public databases for CompoundCrawler to detect them, AnalyteDB empowers users to incorporate their own structures directly on a local level. Sources for such new structures could be for instance deep-learning or AI-based predictions.

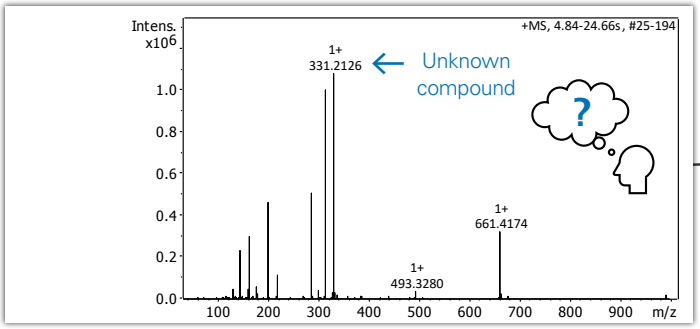
As the final step, the found structure candidates are suspected to *in-silico* fragmentation by **MetFrag** [5] and matched against the experimental MS/MS spectrum. Through the implementation of the *in-silico* fragmentation, the outlined workflow represents a standard-free annotation approach. This is particularly valuable for NPS, where reference standards are often not readily available.

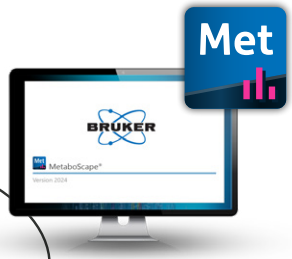
After successful identification of a new compound, it can be added to the spectral library for future comparisons and searches. Furthermore, benefiting from the principle of 'all data is collected all the time', a retrospective evaluation of previously analyzed samples is feasible without the need for repeating any experimental work.

Three-step workflow for the unknown annotation in MetaboScape

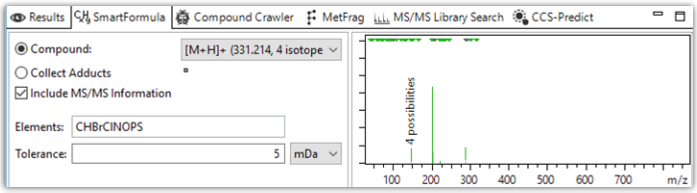
Presented here is the identification of ADB-BUTINACA without the need for a standard.

? Unknown compound





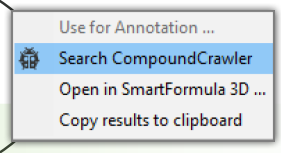
1 SmartFormula



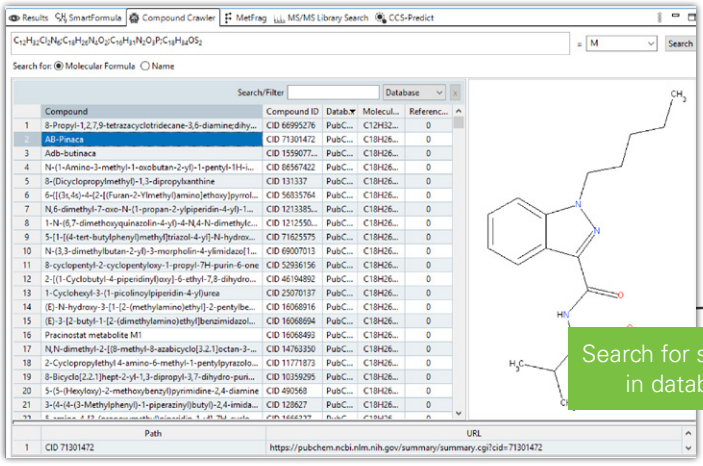
#	Neutral Formula	Ion	m/z calc.	m/z meas.	|Δ m/z| [mDa]	|Δ m/z| [ppm]	mSigma
1	$C_{12}H_{32}Cl_2N_6$	[M+H]+	331.21382687	331.2135	0.3091	0.93	295.84
2	$C_{18}H_{26}N_4O_2$	[M+H]+	331.21285255	331.2135	0.6653	2.01	1.41
3	$C_{16}H_{31}N_2O_3P$	[M+H]+	331.21450632	331.2135	0.9885	2.98	16.40
4	$C_{18}H_{34}OS_2$	[M+H]+	331.21238452	331.2135	1.1333	3.42	51.70
5	$C_{17}H_{31}ClN_2O_2$	[M+H]+	331.21468238	331.2135	1.1646		
6	$C_{10}H_{30}N_6O_4S$	[M+H]+	331.21220110	331.2135	1.3167		
7	$C_{14}H_{34}O_6S$	[M+H]+	331.21488644	331.2135	1.3686		
8	$C_{13}H_{27}ClN_8$	[M+H]+	331.21199704	331.2135	1.5208		
9	$C_{10}H_{31}ClN_6S$	[M+H]+	331.21536834	331.2135	1.8505		
10	$C_{17}H_{30}O_6$	[M+H]+	331.21151514	331.2135	2.0027		
11	$C_{15}H_{38}OS_3$	[M+H]+	331.21575582	331.2135	2.2380		

SmartFormula calculates possible elemental compositions based on the *m/z* measured and fit of isotope pattern

Elemental composition prediction

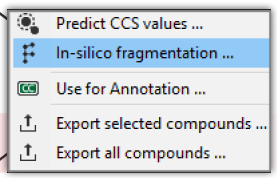


2 CompoundCrawler



CompoundCrawler searches for structure candidates for the proposed elemental compositions

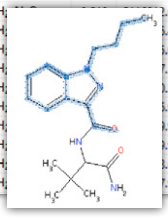
Search for structures in databases



2 MetFrag

Name	Molecular For...	Score	Raw Value	# Frag	IntCov. [%]
Adb-butinaca	$C_{18}H_{26}N_4O_2$	1.0	9872444.855	5	100.0
N-(1-Amino-3-methyl-1-oxobutan-2-yl)-1-pentyl-1H-indaz...	$C_{18}H_{26}N_4O_2$	0.851	8405352.568	5	100.0
AB-Pinaca	$C_{18}H_{26}N_4O_2$	0.851	8405352.568	5	100.0
(2S)-3-methyl-2-([1-pentyl-1H-pyrrolo[2,3-b]pyridin-3-yl]fo...	$C_{18}H_{26}N_4O_2$	0.772	7619037.196	5	100.0
5-amino-4-[3-(propoxymethyl)piperidin-1-yl]-7H-cyclopen...	$C_{18}H_{26}N_4O_2$	0.715	7058864.437	4	98.008
N-[(2R)-1-(dipropylamino)propan-2-yl]-4-(1,2,4-oxadiazol-...	$C_{18}H_{26}N_4O_2$	0.532	5250644.976	4	93.807
4-N,4-N-diethyl-2-N-[4-(3-methoxypropoxy)phenyl]pyrimi...	$C_{18}H_{26}N_4O_2$	0.529	5219044.145	5	100.0
2-Cyclopropylethyl 4-amino-6-methyl-1-pentylpyrazolo[3,4...	$C_{18}H_{26}N_4O_2$	0.523	5159459.371	5	100.0
(E)-N-hydroxy-3-[1-[2-(methylamino)ethyl]-2-pentylbenzi...	$C_{18}H_{26}N_4O_2$	0.521	5145344.743	4	87.109
3-methyl-8-[[(3R,4R)-3-(2-methylpropoxy)piperidin-4-yl]a...	$C_{18}H_{26}N_4O_2$		.637	4	98.008
8-[[1-(3-methoxypropyl)piperidin-4-yl]amino]-3-methyl-1H...	$C_{18}H_{26}N_4O_2$		.409	4	98.008
N-ethyl-4-[2-(2-methoxypropan-2-yl)pyrrolidin-1-yl]-1H-p...	$C_{18}H_{26}N_4O_2$		.003	4	98.008
2-[2-[4-(2-ethoxyethyl)piperazin-1-yl]ethyl]-3H-quinazolin-...	$C_{18}H_{26}N_4O_2$		.177	3	91.814
	$C_{18}H_{26}N_4O_2$		.289	4	
	$C_{18}H_{26}N_4O_2$		.912	2	
	$C_{18}H_{26}N_4O_2$		.953	3	
	$C_{18}H_{26}N_4O_2$		.953	3	
	$C_{18}H_{26}N_4O_2$		.664	3	80.196
	$C_{18}H_{26}N_4O_2$		.901	3	21.796

Ion Formula	m/z meas.	Δ m/z [...	Int.	IntCov. [...
[C12H15N2O2]+	219.114	-0.3	13373.652	2.0
[C12H14N3]+H+	201.121	[-1H] 6.0	41573.344	6.2
[C8H4N2O]+H+	145.041	[-1H] -0.3	86535.080	12.9
[C17H24N3O]+	286.192	0.1	91361.280	13.6
[C12H13N2O]+	201.103	-0.1	438422....	65.3



MetFrag performs *in-silico* fragmentation of the structure candidates and matches them with the experimental MS/MS spectrum

In-silico fragmentation

Differentiation of isomeric synthetic cannabinoids

Distinguishing between isomeric drugs is crucial for accurate identification. While chromatography-free methods don't rely on separation via retention time, DART-QTOF-MS can still effectively differentiate isomeric drugs in numerous instances by analyzing their fragmentation patterns.

ADB-BUTINACA and AB-PINACA are isomeric synthetic cannabinoids sharing the same elemental composition $C_{18}H_{26}N_4O_2$. The structure of these compounds differs only by the position of one methyl-group. This slight structural difference, however, is sufficient to distinguish them by DART-HRMS/MS based on their fragmentation pattern. For ADB-BUTINACA the methyl-group is located at the part of the molecule that generates the neutral loss, whereas for AB-PINACA the methyl-group is located at the detectable fragment ion. A characteristic fragment ion at *m/z* 201 respectively at *m/z* 215 is therefore observed and serves for the differentiation of the two isomers. In the seized paper sample shown in Figure 3, the identification of ADB-BUTINACA is unambiguous due to the detection of the fragment ion at *m/z* 201.

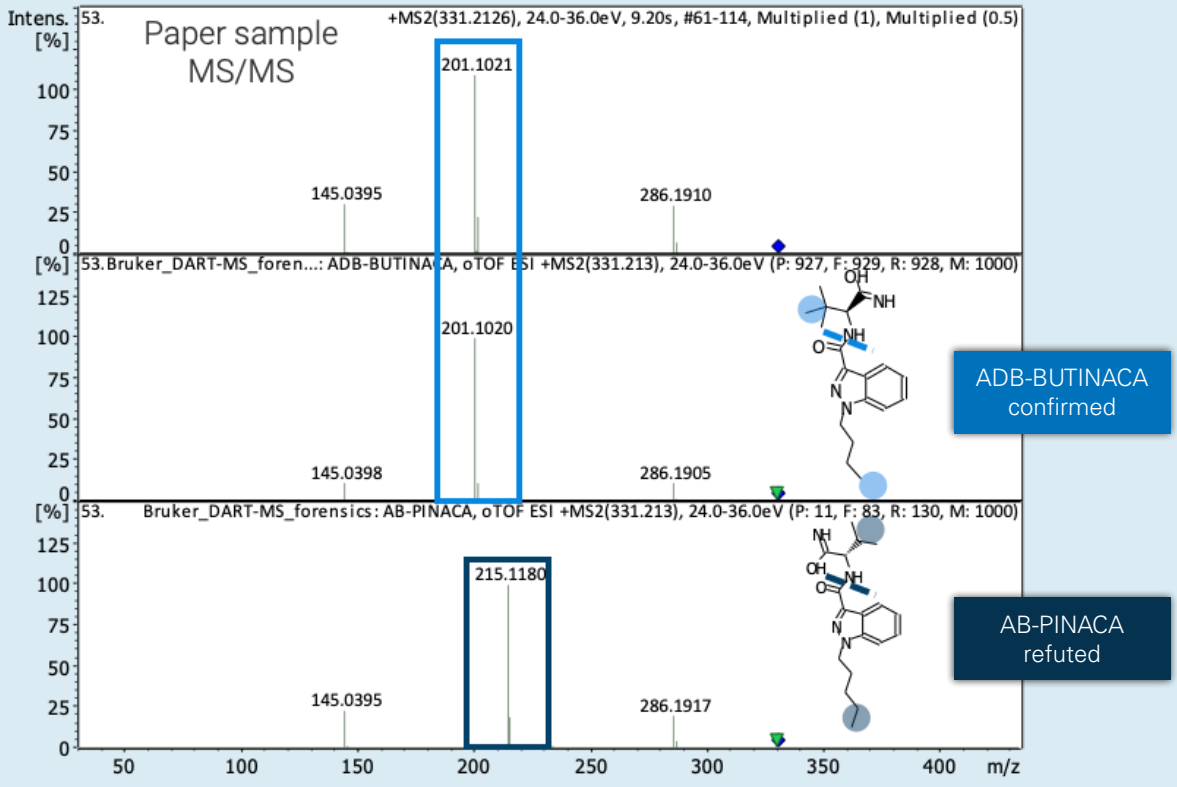


Figure 3. Differentiation of the isomeric synthetic cannabinoids ADB-BUTINACA and AB-PINACA based on their fragmentation pattern. Reference standard based identification of ADB-BUTINACA in an authentic paper sample.

Conclusion

DART-HRMS was demonstrated to be wellsuited for a highly accelerated screening of seized drug samples. It provides a viable alternative to established techniques without the need for time consuming chromatography. Going chromatography-free with DART translates to an immense saving in analysis time per sample to less than a minute as well as a tremendous reduction in solvent consumption. Using Bruker's QTOF mass spectrometer, a high detection selectivity is combined with an excellent sensitivity. It can be applied beyond just targeted analyses as well for the detection of unexpected and the identification of unknown compounds. Since it is operated always in full scan mode, the retrospective analysis of any kind of investigated sample is facilitated, which is particularly desirable in the context NPS.

References

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[3] United Nations Office on Drugs and Crime, Current NPS Threats, Volume VI, August 2023.

[4] Drug Enforcement Administration Special Testing and Research Laboratory, Emerging Threat Report Annual 2022.

[5] Wolf S., Schmidt S., Müller-Hannemann M., Neumann S., In silico fragmentation for computer assisted identification of metabolite mass spectra, BMC Bioinformatics, 2010, 11,148.

Further reading

AppNote AMS-06: Software workflow for identification of unknown NPS

AppNote TN 56: A new paradigm in forensic analysis

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