Technical Note

Forensic Technology
CENTER OF EXCELLENCE
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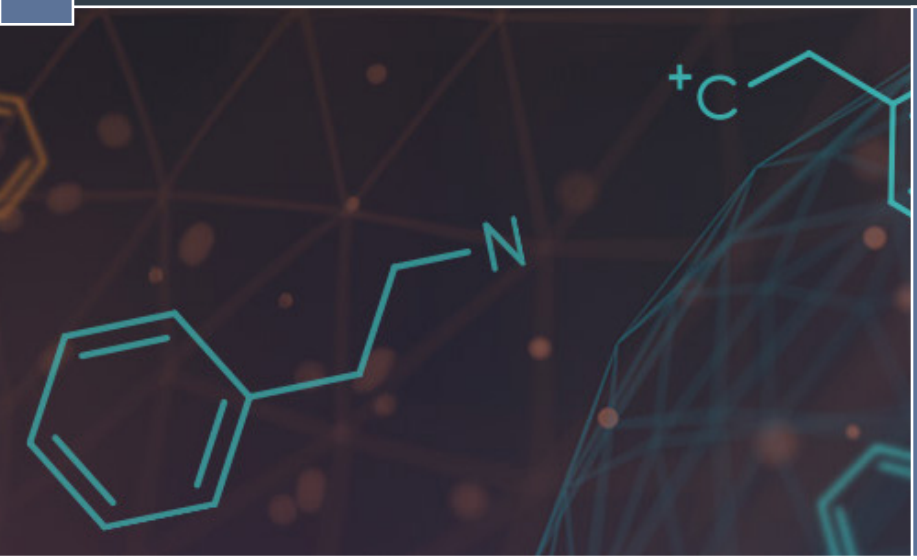
The Use of Neutral Losses and Common Fragments Screening by QTOF in Forensic Laboratories



Technical Contacts

Joshua DeBord, PhD
Center for Forensic Science Research & Education
joshuadebord@cfsre.org

Katherine Bollinger
RTI International
kbollinger@rti.org

Introduction

Quadrupole time-of-flight (QTOF) mass spectrometry is an advanced analytical technique that has some capabilities that make it particularly useful in screening in toxicology cases for unknown analytes. The technology combines the power of a quadrupole mass filter with the resolution and accuracy of TOF analysis. This section describes the process from sample introduction to TOF analysis followed by descriptions of common fragments (CFs), neutral losses (NLs), data analysis, interpretation, and confirmation.

1. **Sample Introduction and Ionization:** The biological sample (e.g., blood, urine) suspected of containing compounds of interest is prepared and introduced into the QTOF instrument. The compounds in the sample are ionized, usually by electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI).
2. **Quadrupole Mass Filtering:** The ions are then passed through a quadrupole mass filter, which can be set to allow only specific precursor ions (based on their mass-to-charge ratio, m/z) to pass through. This selection is useful for targeting specific compounds or classes of compounds.
3. **Collision Cell:** The selected ions are then directed into a collision cell, where they collide with an inert gas. This collision causes the ions to fragment into smaller ions.
4. **TOF Analysis**: After fragmentation, the resulting ions are separated based on their velocities in a TOF analyzer. The TOF measures the time it takes for ions to travel a fixed distance, allowing determination of their m/z values with high precision.
5. **Common Fragments:** Many drugs and their metabolites have characteristic fragments when they are subjected to collision-induced dissociation. By analyzing the fragment ions, you can infer the presence of specific drug compounds. Certain groups of structurally related drugs may produce signature fragment ions that are shared between drugs within the group. These ions can be used as markers for the presence of a drug within the group. For example, a dominant fragment ion for fentanyl, 105.07 m/z, is also a dominant fragment ion in many fentanyl analogs.

6. **Neutral Losses:** Some compounds, when fragmented, lose a specific neutral molecule. By looking for ions that exhibit these characteristic NLs, the presence of certain drug classes or specific drugs can be inferred.
7. **Data Analysis and Interpretation:** Advanced software tools help in processing and interpreting the vast amount of data generated by QTOF. This can involve comparing the observed fragments and NLs to databases containing information about known drug compounds.
8. **Confirmation:** If a compound is presumptively identified in a sample, it is crucial to confirm its presence using a reference standard or by using another analytical method.

Method

Much like other QTOF methods, methods for CF/NL screening are either targeted or untargeted. This section focuses on describing methods of targeted screening and untargeted screening and methods of inputting CF/NL into processing by batch or individual sample.

1. **Targeted Screening:** The instrument is set to look for specific fragments or NLs known to be associated with certain compounds or classes of compounds. For example, if only interested in compounds that produce a triethylamine cation, you would set the instrument to monitor that specific fragment. This type of screening is usually done to address a known deficiency or blind spot in current methods.
2. **Untargeted Screening:** The instrument monitors for a wide range of potential CFs and NLs without a predefined list. Once the data are acquired, you would then process it to identify which CFs or NLs are present and potentially identify or categorize the compounds based on those observations. This type of screening is more general and could even be applied as a data-processing method only, given the proper acquisition mode is used. This can be achieved by using untargeted scanning modes, which can be either data-dependent or data-independent. Some examples of these modes include information-dependent data acquisition [Sciex], MSe [Waters], or Auto MS-MS [Agilent]. The MS/MS fragments must be associated with the parent HRMS ion for the data analysis software to calculate the neutral loss.
3. **CF/NL Expected Mass Input:** In practice, CFs and NLs are typically input as an expected mass and a threshold of tolerance, but data applications execute the mass filter in different ways. Some software allows for CF/NL methods to be set up as a processing method, like a library search, which can be applied across multiple samples for a batch analysis. Others are more rudimentary and are entered on demand and processed for individual samples. Inquire with prospective vendors about the capabilities and functionalities of their systems and software regarding CF/NL screening.
4. **CF/NL Input Formula:** Recall when entering values for the processing method that NLs are neutral species and CFs are charged species, usually protonated, but can be sodium-adduction, potassium-adducts, or other ion types. However, they are generally inputted as Da values of their $(M+X)^+$ formula.
5. **Example CF/NL:** The data presented in this guide pertain to positive ionization mode. Negative ionization mode can be used, but the CFs and NLs will be different than those presented here. In ESI negative ion mode, NL screening can be performed, but it typically focuses on losses that are relevant to molecules that form anions easily:
   a. Loss of Water (Δ18 Da)**:** This is a common NL for many organic molecules undergoing deprotonation, as they can lose a water molecule (H_2O).
   b. Loss of Carbon Dioxide (Δ44 Da)**:** Molecules with carboxylic acid groups may lose CO_2 upon collision-induced dissociation.
   c. Loss of Sulfur Compounds: For example, loss of sulfur dioxide (SO_2, Δ64 Da) is common in compounds containing sulfonyl groups.
   d. Phosphoric Acid Loss: In the analysis of phospholipids or phosphorylated compounds, the loss of phosphoric acid (H_3PO_4, Δ98 Da) is a characteristic NL.
   e. Loss of Halides: Molecules that contain halogens (e.g., chlorinated or fluorinated compounds) may lose a halide ion (Cl-, F-).

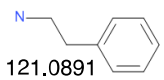
Resources

The CFs and NL masses must be known or anticipated to apply them as a mass filter. Therefore, prior knowledge is required. The best resources for CF/NL information are HRMS libraries. This resource contains a modified version of CFSRE's Neutral Loss and Common Fragments LIst with calculated fields for CFs and NLs rounded to 10 mDa. Novel psychoactive substances (NPS) are a challenging category of analytes in forensic casework, which can be screened by CFs and NLs. Figures 1–3 detail some of the relevant CFs and NLs for some sub-groups of NPS compounds (e.g., fentalogs) taken from the modified library. The rows show the proposed structures of the highlighted fragments. The blue rows in the figure on the left emphasize common structures among several compounds within the drug class, whereare the orange rows in the figure on the right emphasize fragments that may only be useful for one or a few compounds. The letter "n" is number of compounds within the drug class in CFSRE's library that are found to produce the given HRMS fragment/calculated NL. Coverage is the proportion of compounds in the drug class (in CFSRE's library) that has the given HRMS fragment/calculated NL. In other words, CF/NL with higher coverage will be more useful for screening for a class of compounds. Although the library is available as a resource, it is constantly updated and may contain additional compounds from when the figures below were created.

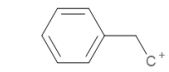
Figure 1: CFs and NLs for fentalogs (i.e., fentanyl analogs)

Fentalog (n=127)

NL			CF		
m/z	n	Coverage	m/z	n	Coverage
116.0473	5	4%	84.0807	5	4%
121.0891	65	51%	105.0699	84	66%
149.0841	28	22%	132.0807	6	5%
232.1576	19	15%	134.0964	38	30%
			140.107	2	2%
			146.0964	28	22%
			174.1277	6	5%
			188.1434	79	62%
			216.1383	11	9%
			245.1648	3	2%
			246.1489	5	4%
			281.2012	11	9%



121.0891



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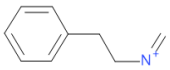

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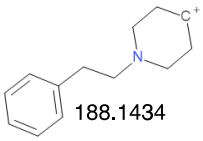

188.1434

Fentalog (n=127)

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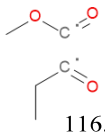
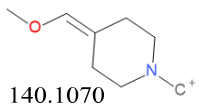
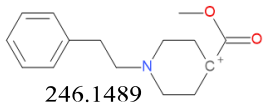


116.0473



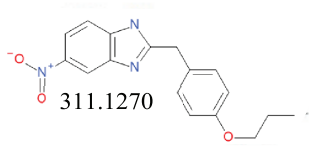
140.1070

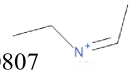


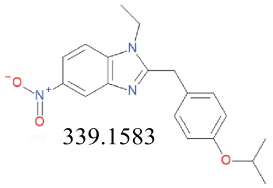
246.1489

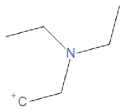
Nitazene (n=32)

NL			CF		
m/z	n	Coverage	m/z	n	Coverage
297.1113	6	19%	44.0495	23	72%
311.1270	8	25%	69.0699	2	6%
339.1583	9	28%	72.0807	27	84%
			100.1121	25	78%
			107.0491	14	44%









Nitazene (n=32)

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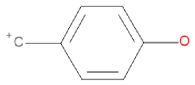


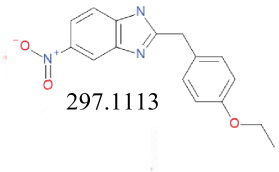
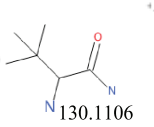
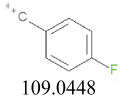


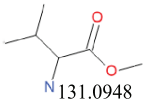
Figure 2: CFs and NLs for nitazene analogs

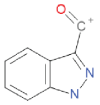
Synthetic Cannabinoids (n=149)

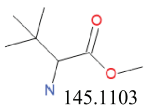
NL			CF		
m/z	n	Coverage	m/z	n	Coverage
45.0209	41	28%	77.0386	6	4%
130.1106	21	14%	86.1000	4	3%
131.0946	19	13%	93.0699	8	5%
135.1048	12	8%	105.0335	6	4%
145.1103	25	17%	107.0855	13	9%
259.1321	6	4%	109.0448	22	15%
			116.0495	20	13%
			119.0855	14	9%
			135.1168	13	9%
			144.0444	28	19%
			145.0396	47	32%
			177.0500	10	7%
			189.0459	4	3%
			212.1070	11	7%
			213.1022	17	11%
			222.9500	4	3%
			241.1335	10	7%
			253.0800	11	7%

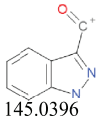












Synthetic Cannabinoids (n=149)

NL			CF		
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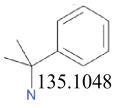
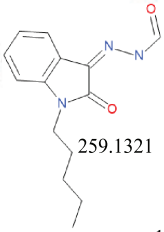
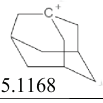
135.1048	12	8%	105.0335	6	4%
145.1103	25	17%	107.0855	13	9%
259.1321	6	4%	109.0448	22	15%
			116.0495	20	13%
			119.0855	14	9%
			135.1168	13	9%
			144.0444	28	19%
			145.0396	47	32%
			177.0500	10	7%
			189.0459	4	3%
			212.1070	11	7%
			213.1022	17	11%
			222.9500	4	3%
			241.1335	10	7%
			253.0800	11	7%







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FTCOE Contact
Jeri Ropero-Miller, PhD, F-ABFT
Principal Scientist, FTCOE
jerimiller@rti.org

NIJ Contact
Danielle McLeod-Henning, MFS
Physical Scientist
Office of Investigative and Forensic Sciences
danielle.mcleod-henning@usdoj.gov

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