Detection of Designer Benzodiazepines via Mass Spectrometry-Based Molecular Networking

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Background

Drug-facilitated sexual assault (DFSA) poses a significant concern due to the widespread use of 'date-rape' drugs such as designer benzodiazepines (DBZDs). Forensic detection of these drugs is difficult as minor chemical modifications render them undetectable to traditional targeted drug tests. Molecular networking offers a spectral similarity-based technique that organizes MS/MS spectra into molecular 'families', enabling detection and subsequent clustering of unknown, but chemically related compounds. It is hypothesized that the MS/MS spectra of BZDs and designer analogues can be detected and networked via an open-source cosine similarity score-based algorithm called GNPS.

Benzodiazepines

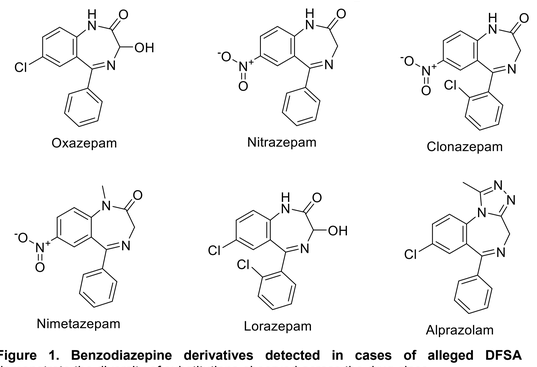

Figure 1. Benzodiazepine derivatives detected in cases of alleged DFSA demonstrate the diversity of substitutions observed across the drug class.

Molecular Networking

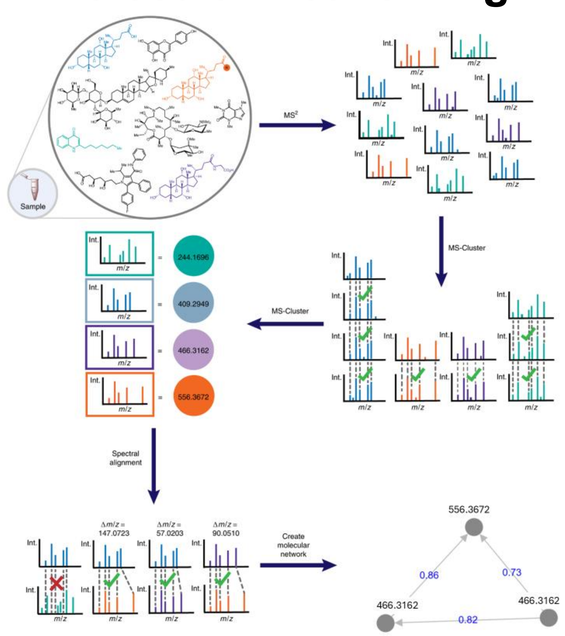

Figure 2: Molecular Networking General Workflow. Figure adapted from reference 1.

Cosine-Similarity Scoring

Global Natural Products Social Molecular Networking Platform (GNPS) uses a modified cosine similarity algorithm to evaluate similarity between spectra.

Cosine similarity scoring calculates the normalized dot product between two vectors which represent two MS/MS spectra, calculated using the equation below.

GNPS' modified cos score calculation also allows for mass-shifted peak alignment, meaning fragment ions can still be calculated as matches if they differ by a consistent precursors mass of known chemical modifications (ie. halogenation, dehydration, etc). This allows for recognition of structurally related molecules (like drug analogs) that share similar fragmentation patterns but differ in mass due to metabolism-induced chemical modifications.

$$Similarity(S,S') = \frac{\sum_{i=1}^{t} s_i \cdot s'_i}{\sqrt{\sum_{i=1}^{t} s_i^2 \cdot \sum_{i=1}^{t} s'^2_i}}$$

Equation 1: Cosine similarity score is calculated between two spectra (S and S') where s_i represents the intensity of a fragment and t is the sum of two sets of compared masses. From reference 2.

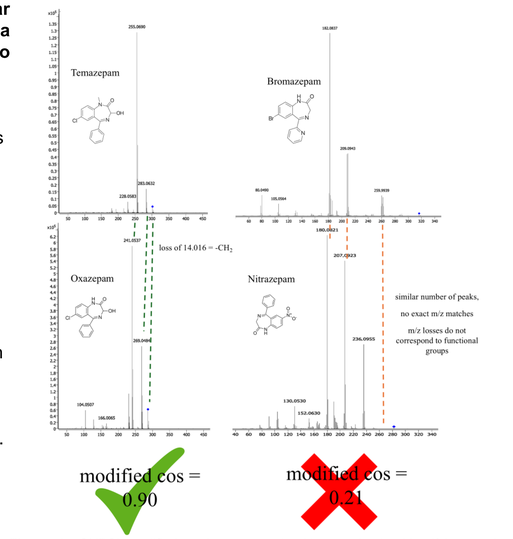

Figure 3: GNPS' modified cosine score algorithm works by identifying peaks between spectra with identical m/z's and/or peaks with m/z differences that match common functional groups. A cos score of 0 represents no matched peaks and highly different spectra, while a score of 1 indicates 2 identical spectra.

Network Results

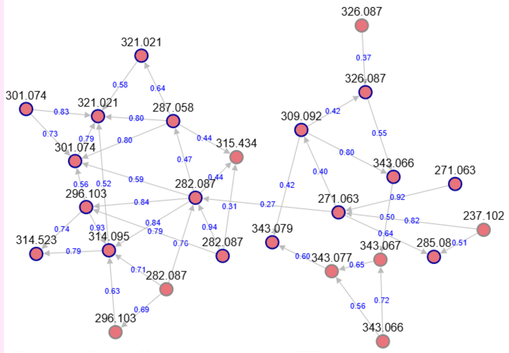

Figure 4: Classic Molecular Network of BZDs with Human Serum Compounds.

BZD Network has no 'false positive' connections to human serum metabolites even with low (0.2) cos score.

This network was generated using MS/MS spectra from all purchased BZDs and 3 human serum samples

Results demonstrate that BZDs yield significantly unique MS/MS spectra compared to serum metabolites.

Results suggest that molecular networking *is* an applicable technique in complex biological matrices.

Feature-Based Molecular Networking (FBMN) can detect BZDs with low cosine scores and link them together.

GNPS successfully linked 15/17 BZDs and ½ BZD core structures together, even with a very low cos score (0.2).

BZDs with highly different structures (ie. etizolam and triazolam) still linked as 'similar', with low cos scores (0.29), while near-identical drugs like temazepam and lorazepam were linked with a score of 0.92.

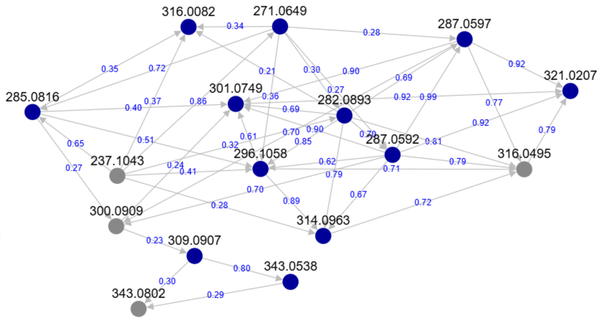

Figure 5: FBMN of only BZDs shows connections to other BZDs with large structural differences and thus low modified cosine similarity scores.

Forensic Results

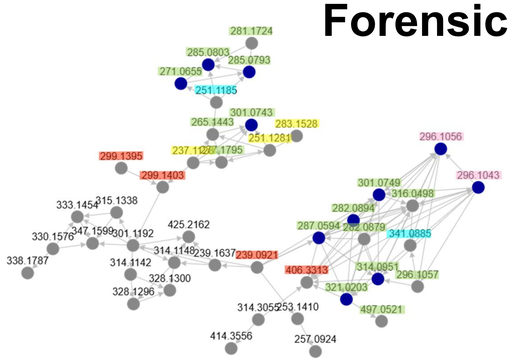

Figure 6: FBMN showing connections between nimetazepam spiked in human serum samples and a network of BZDs. Nodes represent individual features, edges represent a connection with a cos score >0.2. Nodes highlighted in green are BZDs, pink are nimetazepam, blue are undetermined LC column contaminants, and red are non-BZD serum compounds that 'break; the network and lead to further connections with non-BZD compounds.

BZD Network can recognize a novel DBZD spiked in human serum sample as a related compound.

Input spectra included human serum sample spiked with nimetazepam and every other previously run BZD (excluding nimetazepam).

Some false positives (red links) occurred but had low cos scores (0.43-0.53) and were single links.

Glucuronidated lorazepam was linked to BZD network, suggesting that BZD metabolites can also be recognized by molecular networking.

Future Work

- BZD metabolite generation via liver microsomal assays to mimic human drug metabolism will provide additional spectra of metabolized BZDs for network inclusion
- Molecular networking of BZD metabolites will increase applicability to true forensic cases
- Switching from GNPS to a more drug/metabolite specific software may decrease processing speed
- Streamlining LCMS/MS method and downstream molecular networking protocol for forensic laboratories

The underlying analytical detection methods explored in this study are chemically interesting and provide insight towards the future of mass spectrometry data analysis. However, **future work should continue to emphasize translating these findings into improved legal frameworks and expanded support for victims, ensuring that scientific advances ultimately contribute to greater justice and protection of victims of sexual assault.**

Summary of Findings

- MS/MS spectra were generated on a LC-Q-ToF mass spectrometer for a collection of chemically diverse legal and illegal BZDs.
- Modified cosine-similarity score-based molecular networking linked the MS/MS spectra of a range of BZDs to each other and recognized them as spectrally similar.
- There was no overlap (false-positives) between MS/MS spectra of BZDs and human serum samples, indicating MN can recognize BZDs as structurally distinct from endogenous serum compounds.
- An illegal BZD analogue spiked into a human serum sample was linked to other BZDs with high cos scores (0.52-0.9), demonstrating the forensic potential of molecular networking in true cases of DFSA.

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Works Cited

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