Application of Structure-Based LC/MS Database Management for Forensic Analysis

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Overview

- Application of LC/UV/MS to the analysis of toxicologically relevant samples
- Demonstration of several ways in which structural information may assist in sample identification
  - Use of a structure-based data management system for manual interpretation and analysis of sample components
  - Software tools to interpret difficult analysis cases (e.g., high noise backgrounds, manual and automated fragment assignment tools)

Introduction

- In many cases, an automated search method can be used for sample identification
  - However...
    - Automated search results still must be manually reviewed
    - Difficult or low concentration samples require significant manual interpretation
    - Commercially available databases are only available with nominal mass accuracy
- Integration of structures with spectral information can provide improved data management and enhance library searching capabilities
  - Particularly useful for manual interpretation and analysis of results
  - Provides structure and substructure search capabilities for data mining
  - Allows management of multiple forms of MS data with a single chemical structure – EI, ESI, CI, etc.

LC/UV/MS Conditions

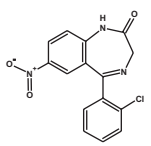
- Chromatography
  - Instrument: Waters 2690 Alliance™ System under MassLynx control
  - Solvents: A: H_2O, B: MeCN
    - 200 µl/min
    - 30-40% B in 20 min
    - 35 min total run time
  - 2.1x100 mm XTerraMS™ C_{18} 3.5 µm
    - 35 C column temp
- PDA
  - Instrument: Waters Model 996 Photodiode Array Detector
  - 210-400 nm
- Samples
  - Standards diluted in 30/70 MeCN/H_2O
- Mass Spectrometry
  - Instrument: Waters ZQ™ 4000 under MassLynx control
  - ESI+, centroid
  - Scan 100-600 amu
  - Scan time: 0.2 sec
  - Interscan delay: 0.1 sec
  - Tune page settings
    - Capillary voltage: 3.0 kV
    - Cone voltage: 30, 50, 70 V
    - Extractor: 6 V
    - RF lens: 0.1 V
    - Ion energy: 0.1
    - LM & HM resolution: 14.5 &15.0
    - Source temperature: 150 C
    - Desolvation temperature: 300 C
    - Cone gas: 150 l/hr
    - Desolvation gas: 600 l/hr

Sample Preparation Procedure

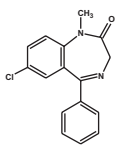
for Spiked Samples

30 mg Waters Oasis HLB Cartridge
Condition
1 ml MeOH and 1 ml H_2O
Load
1 ml spiked serum or Hanks' solution (10:1 dilution of standards with serum or Hanks')
Wash
1 ml 5% MeOH in H_2O
Elute
1 ml MeOH
Evaporate and Reconstitute
Room temp in Savant Speed Vac 200 µl mobile phase (30% MeCN in H_2O)

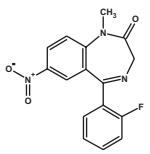
Benzodiazepines



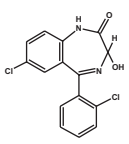
Clonazepam
315.0411 m/z



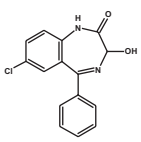
Diazepam
284.0716 m/z



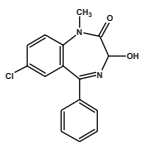
Flunitrazepam
313.0863 m/z



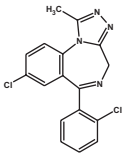
Lorazepam
320.0119 m/z



Oxazepam
286.0509 m/z



Temazepam
300.0666 m/z

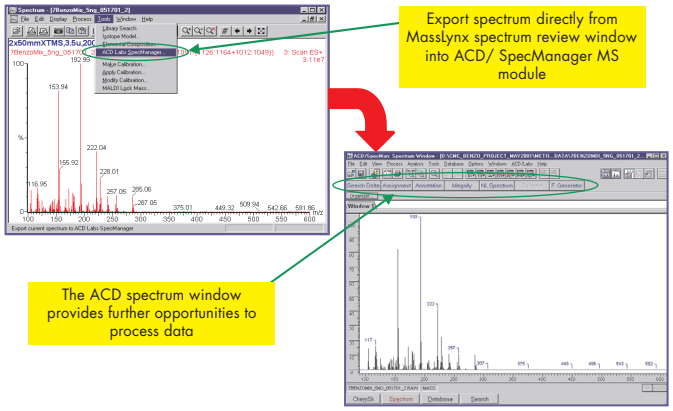


Triazolam
342.0439 m/z

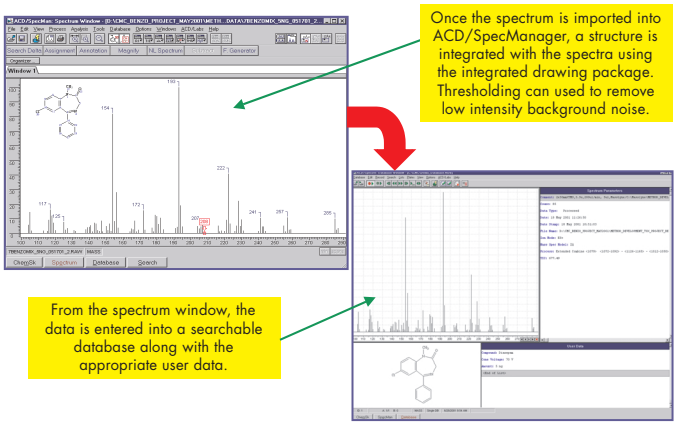
Monoisotopic Masses

Creating UV and MS Libraries

Export Data from MassLynx into ACD



Add Spectrum to Database



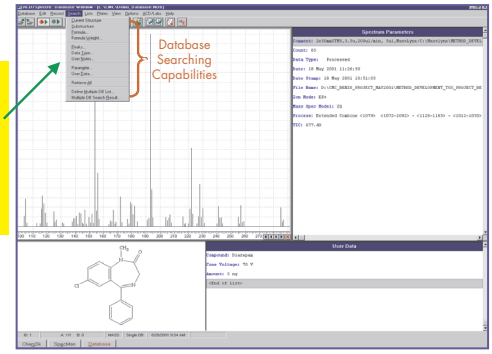
Creating a UV and MS Database

- Individual benzodiazepine standards were analyzed under the conditions given previously
  - All MS data (3 cone voltages used to enhance descriptive fragmentation for each compound) were entered into one comprehensive database using the procedure presented below
  - Following a similar procedure, UV data were also incorporated into the database
- Chemical structures were incorporated into both the UV and MS library entries using the integrated drawing package

Manually Searching Data in the Database

Once the database is populated, there are several ways in which the data may be accessed and searched.

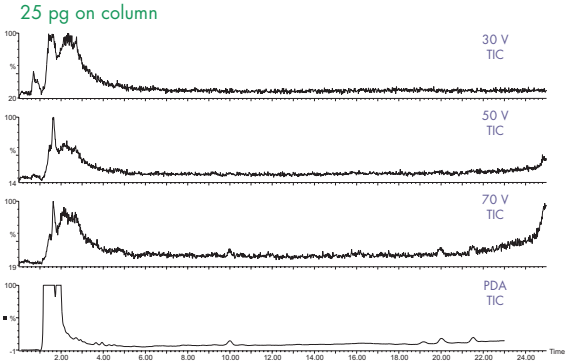
- Ways to search data
  - By spectra
  - By structure
  - By substructure
  - By individual fragment peaks
  - By user-defined text
  - By single DB or multiple DB



Application of Structure-Based LC/MS Database Management for Forensic Analysis

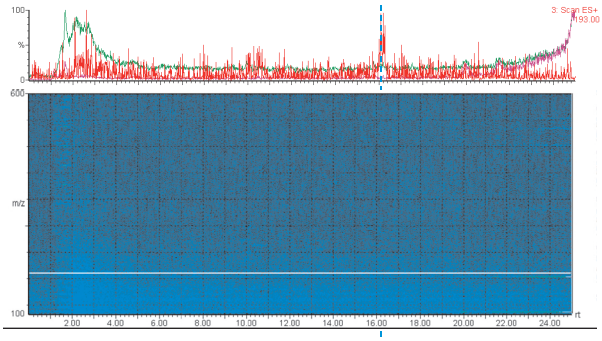
Manual Interpretation of Low Concentration Samples

Little Information Available from TICs at Low Concentrations



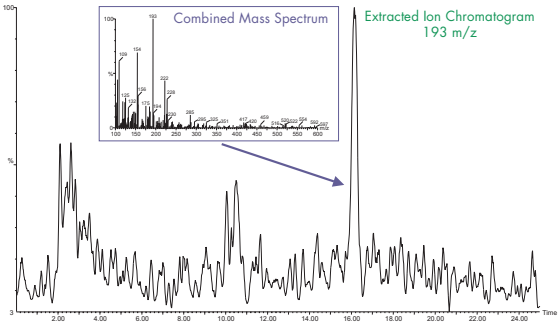
Total ion current chromatograms (TIC) produced in the analysis of spiked serum (25 pg of each benzodiazepine on column). Little information is gained from TICs. By visualizing data with the Map function in MassLynx, it is possible to pick peaks out of the noise.

Pulling Peaks Out of the Noise



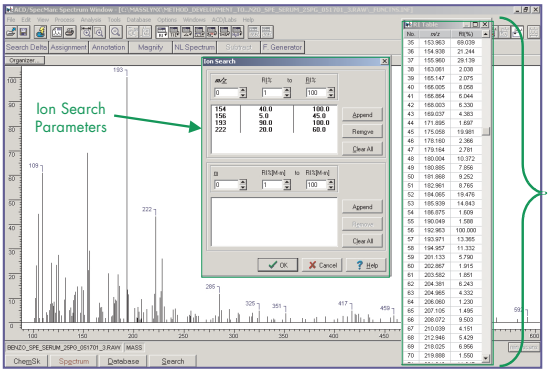
Using the Map function in MassLynx, it is possible to manually scroll through individual m/z ratios collected during a scan run for each function. Through this functionality, peaks may be picked out that are not evident in the TICs. For example, with a cone voltage at 70 V, a peak at 193 m/z was found around 16 minutes.

Extraction of "Hidden Peaks"



Once individual masses are distinguished from the noise, extracted ion chromatograms can be created and used to generate mass spectra. The mass spectra can then be searched against MS libraries for identification. However, if the noise is high, extraneous peaks may diminish the utility of automated searches.

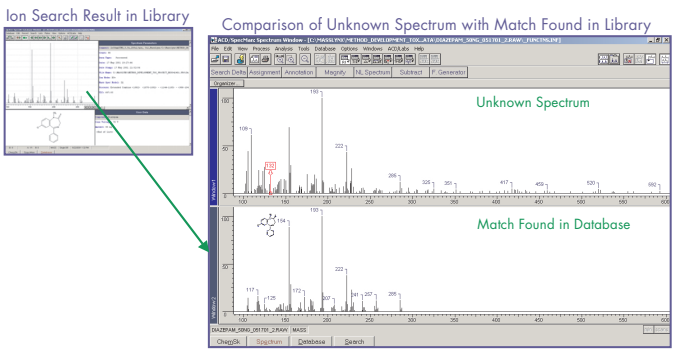
Ion Searches



In cases where manual interrogation of data is necessary, software that facilitates the process can translate into great time savings. ACD software provides the ability to easily ascertain the relative intensities of all the peaks and perform searches against MS libraries of selected peaks based on their relative intensities.

Ion Search Results

The manual selection of peaks and use of relative intensities for searches allows otherwise "unmatchable" spectra to be found in the database. Manual comparison of the library spectrum from the database and the unknown spectrum is then used to assist in the determination of the identity of the compound.



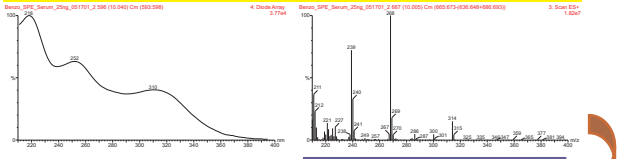
Application of Structure-Based LC/MS Database Management for Forensic Analysis

Using Multi-Spectral Data

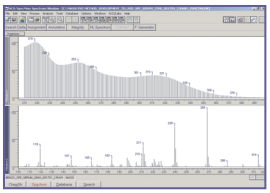
Export Data from MassLynx into ACD

The use of multi-spectral data can provide multiple reference points from which to verify compound identity. This can lead to increased confidence in results. Here we show how multi-spectral data can be used with the structure-based database.

PDA and MS Data from MassLynx



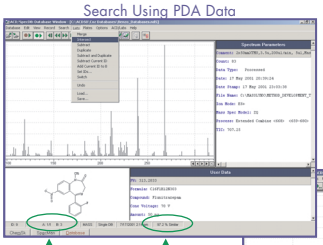
Data is exported out of MassLynx into ACD/SpecManager. Thresholding is used to remove extraneous background peaks from the MS spectrum.



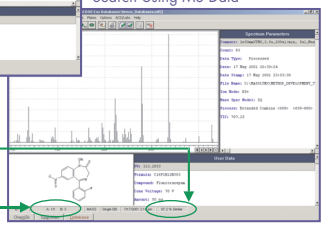
LC/UV/MS run: 25 ng of each benzodiazepine standard on column

Searching the Database with Multi-Spectral Data

Search Using PDA Data

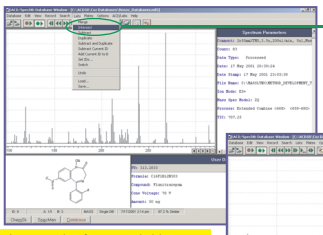


Search Using MS Data

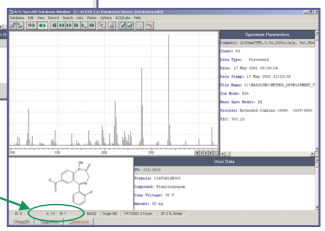


Similarity between unknown spectrum and library spectrum

Number of library matches PDA matches reflected in List A MS matches reflected in List B



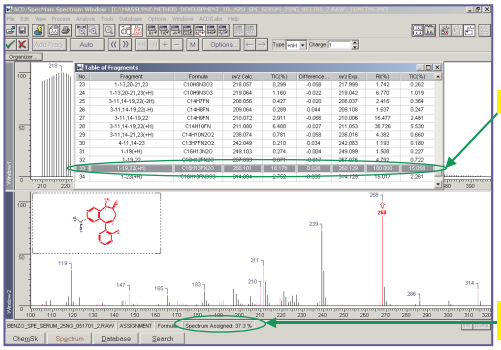
Intersect List A and List B to confirm matching hits from the PDA and MS searches



1 overlapping hit from each library: Flunitrazepam at 70 V

Autofragmentation

The identified MS spectrum may be further evaluated using ACD/SpecManager's Autofragmentation algorithm. Using a proposed structure, the software fragments the molecule and compares the fragments to the spectrum.



Proposed fragment

Percentage of spectrum assigned fragments

Summary

- Structure-based LC/MS database can provide useful tools for the analysis of unknown samples
  - Peak searching for samples containing high background levels against commercial or in-house databases
  - Library searching of both UV and MS spectra; higher degree of confidence when orthogonal results coincide
  - Autofragmentation process assists in the analysis of fragment ions in MS spectra
  - Chemical structure and substructure searching using prior knowledge can provide a means by which to analyze spectra