Improved Sample Identification and Characterization of Toxicological Samples Using Integrated Multi-Spectral Data

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Typical Toxicology Labs

- GC/MS is the method of choice
  - Time honored
  - Ease of use
  - Widely accepted methods and libraries
  - Works well for volatile components
  - Relatively inexpensive instrumentation costs

Potential of LC/MS in Tox Labs

- Large percentage of toxicological samples can be analyzed by LC
  - Ability to separate compounds with a wide range of physical and chemical characteristics
- Good alternative for the analysis of polar, high molecular weight, thermally labile, and semi- and nonvolatile compounds
  - Beta blockers, certain benzodiazepines
- Need for sample derivatization greatly reduced

Complementary to GC/MS analyses

Benefits of Multispectral Data

- Improved detection
  - Compounds may be more amenable to one mode of detection than another
    - Compounds that do not have strong chromophores
    - Compounds that do not electrospray well
  - Interferences from the matrix may be seen in one detection scheme but not another
- Multiple reference points for verification from one sample run
  - PDA
    - Distinctive absorption characteristics
  - MS with collision induced dissociation (CID)
    - Molecular and fragment ion information

Instrument Schematic and Available Information

Polarity Information

Retention Time

Absorption Information

UV Spectra

Mass Information

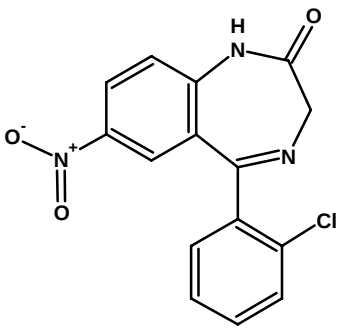
Parent Ion

Cone Voltage 30 V

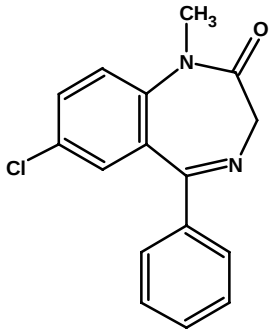
Fragment Ions

Cone Voltage 50 & 70 V

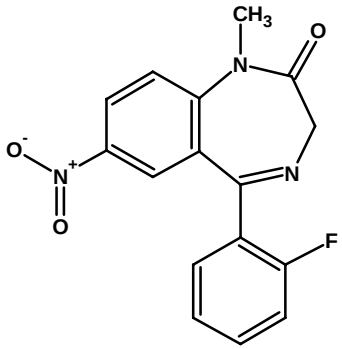
Benzodiazepines



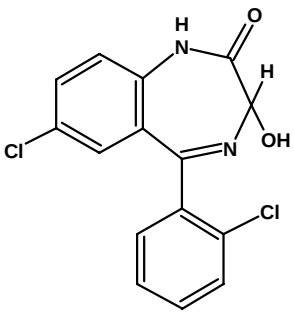
Clonazepam
315.0411 m/z



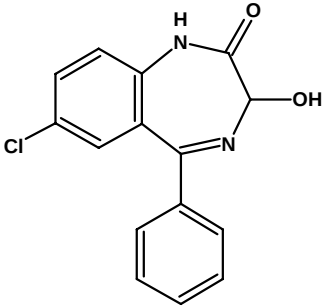
Diazepam
284.0716 m/z



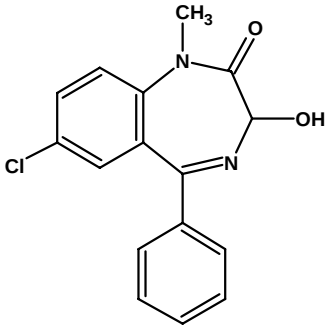
Flunitrazepam
313.0863 m/z



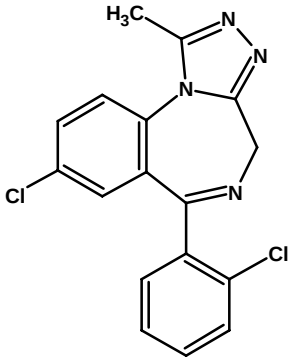
Lorazepam
320.0119 m/z



Oxazepam
286.0509 m/z



Temazepam
300.0666 m/z



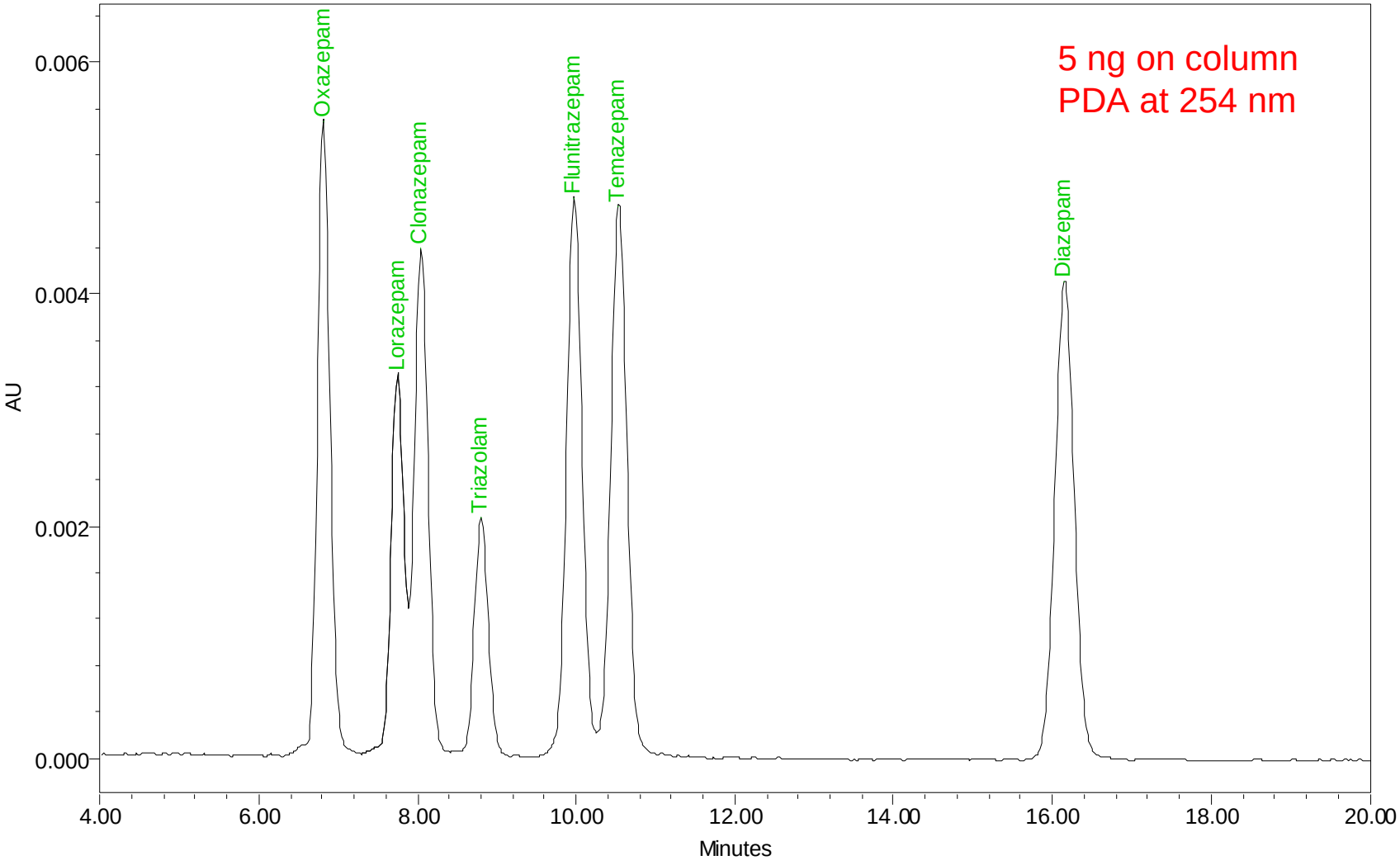
Triazolam
342.0439 m/z

Monoisotopic Masses

LC/UV/MS Conditions

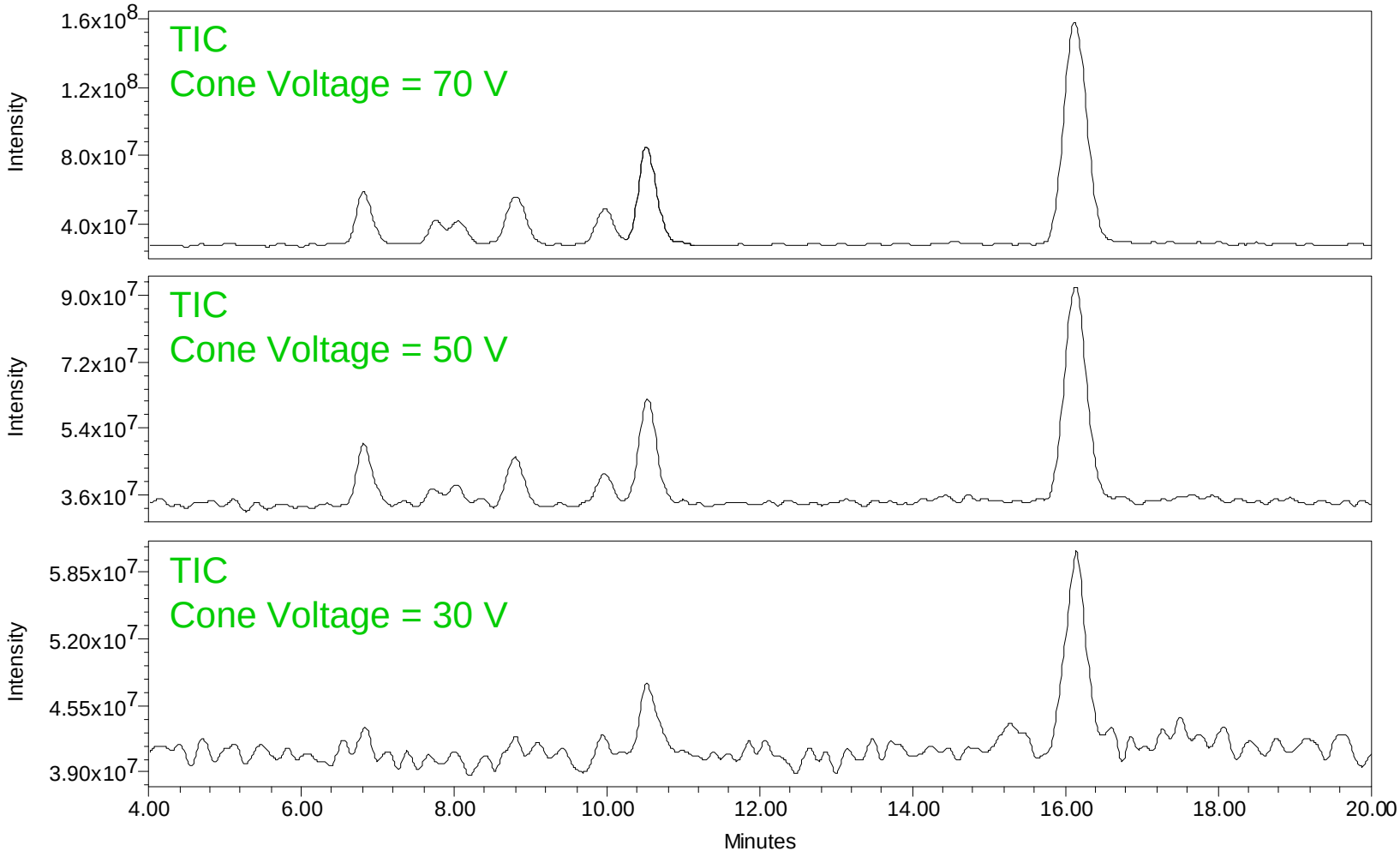
- Chromatography
  - Instrument: Waters 2690 Alliance™ System under Millennium v. 4.0
  - Solvents: A: H_2O, B: MeCN
    - 200 µl/min
    - 30-40% B in 20 min
    - 35 min total run time
  - 2.1x100 mm XTerraMS™ C_{18}, 3.5 µm
    - 35 C column temp
- PDA
  - Instrument: Waters 996 Photodiode Array Detector
  - 210-400 nm
- Mass Spectrometry
  - Instrument: Waters ZQ™ 4000 under Millennium v. 4.0
  - 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

Benzodiazepine Standards



TICs of Standards at Various Cone Voltages

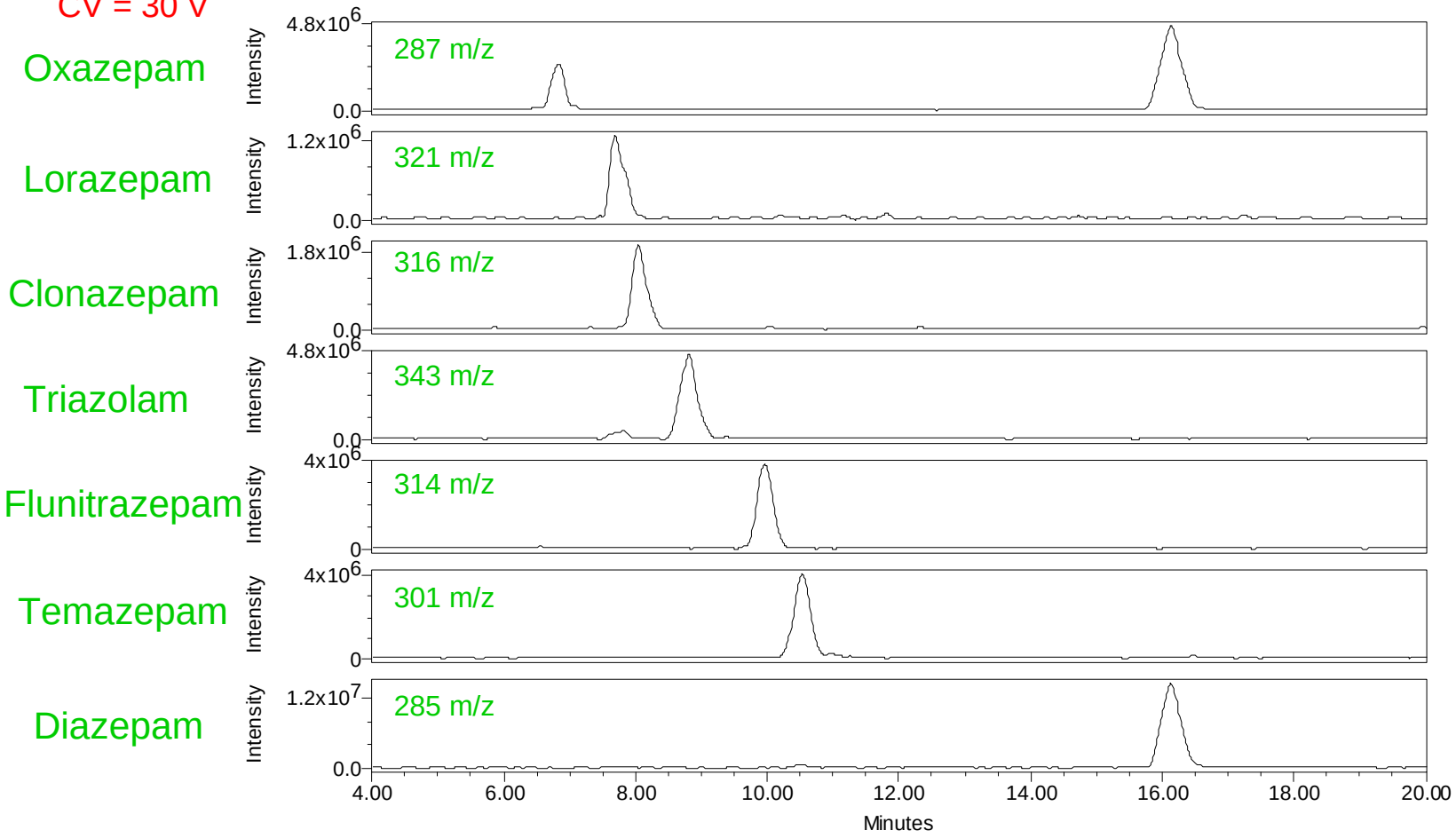
5 ng on column



Extracted Ion Chromatograms of Standards

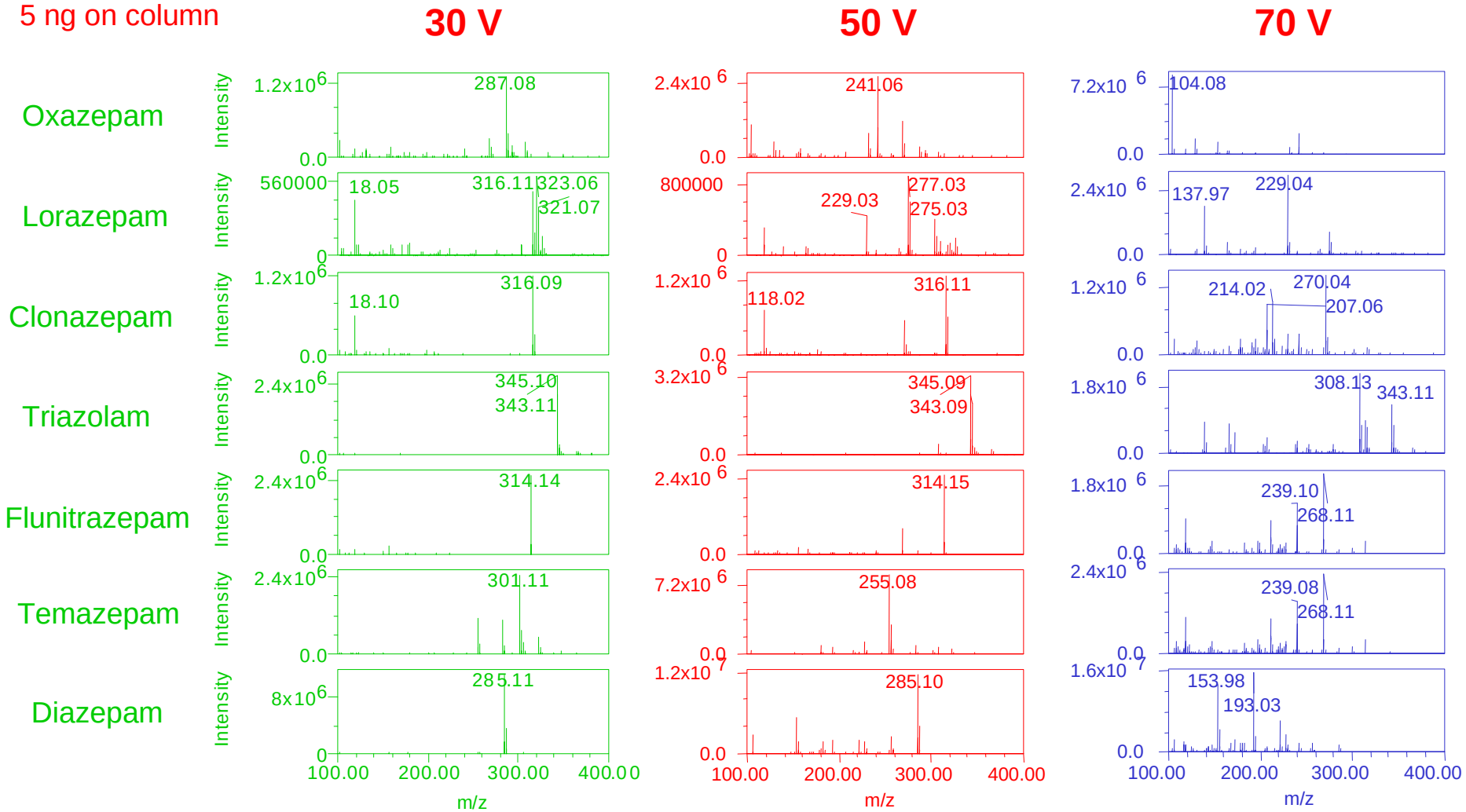
5 ng on column
CV = 30 V

Extracted and Smoothed Ion Chromatograms



Mass Spectra of Standards at Various Cone Voltages

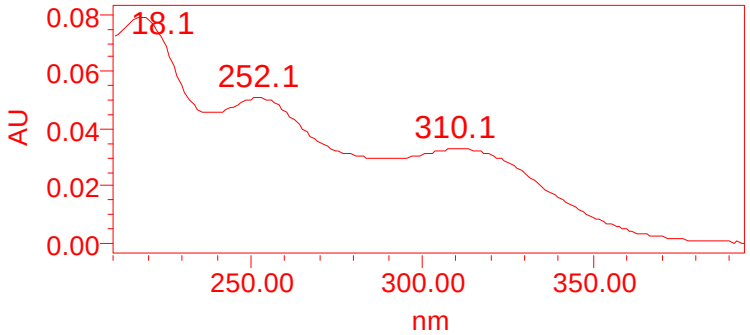
5 ng on column



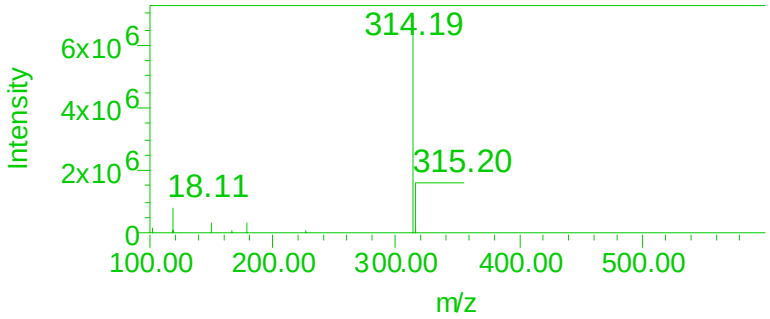
Library Spectra

Searchable Libraries

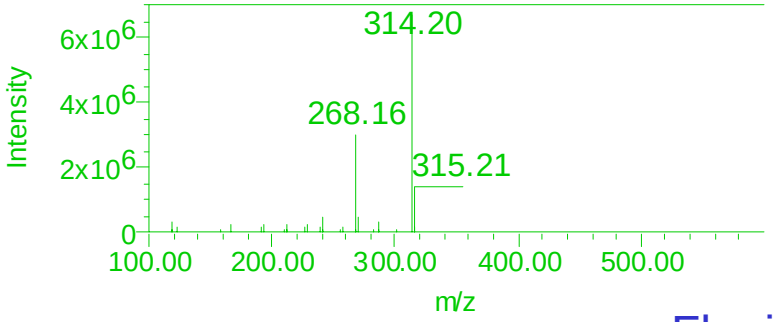
PDA: 210-400 nm



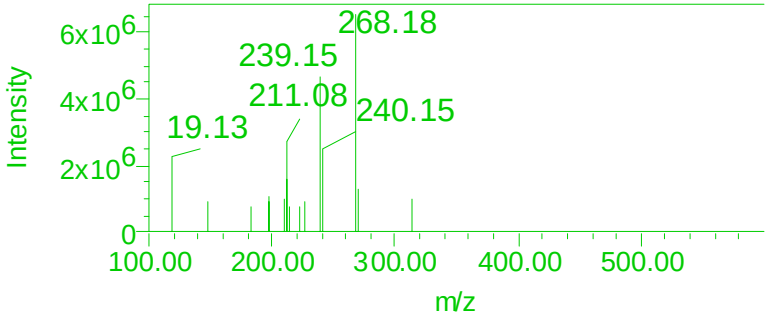
MS: Cone Voltage = 30 V



MS: Cone Voltage = 50 V



MS: Cone Voltage = 70 V



Flunitrazepam

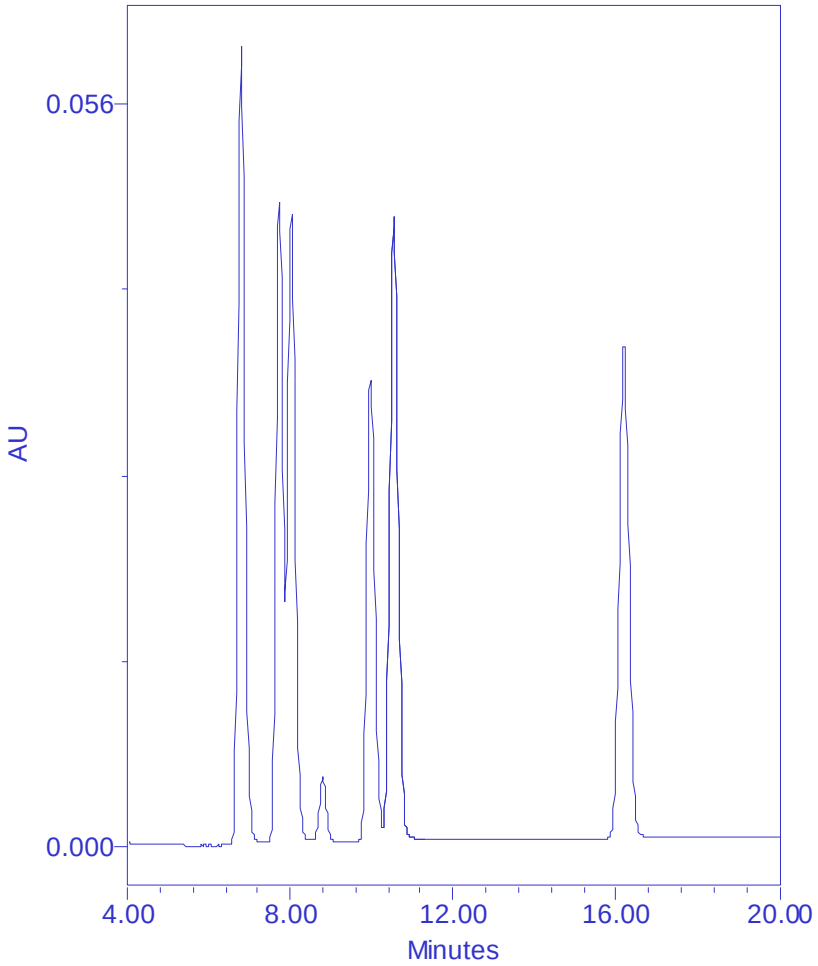
Plus Retention Index

Extraction Procedure for Serum and Hanks' Solution

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)

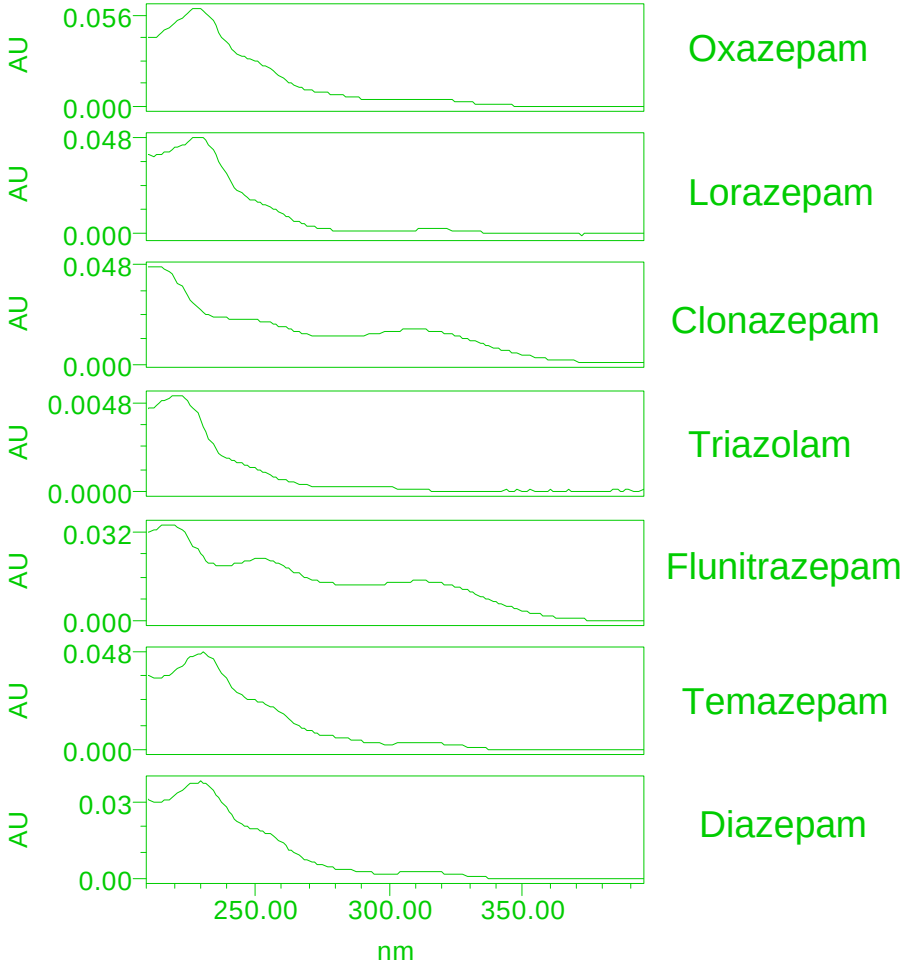
Serum Extract

Total Absorbance Chromatogram



PDA Spectra

25 ng on column
210-400 nm

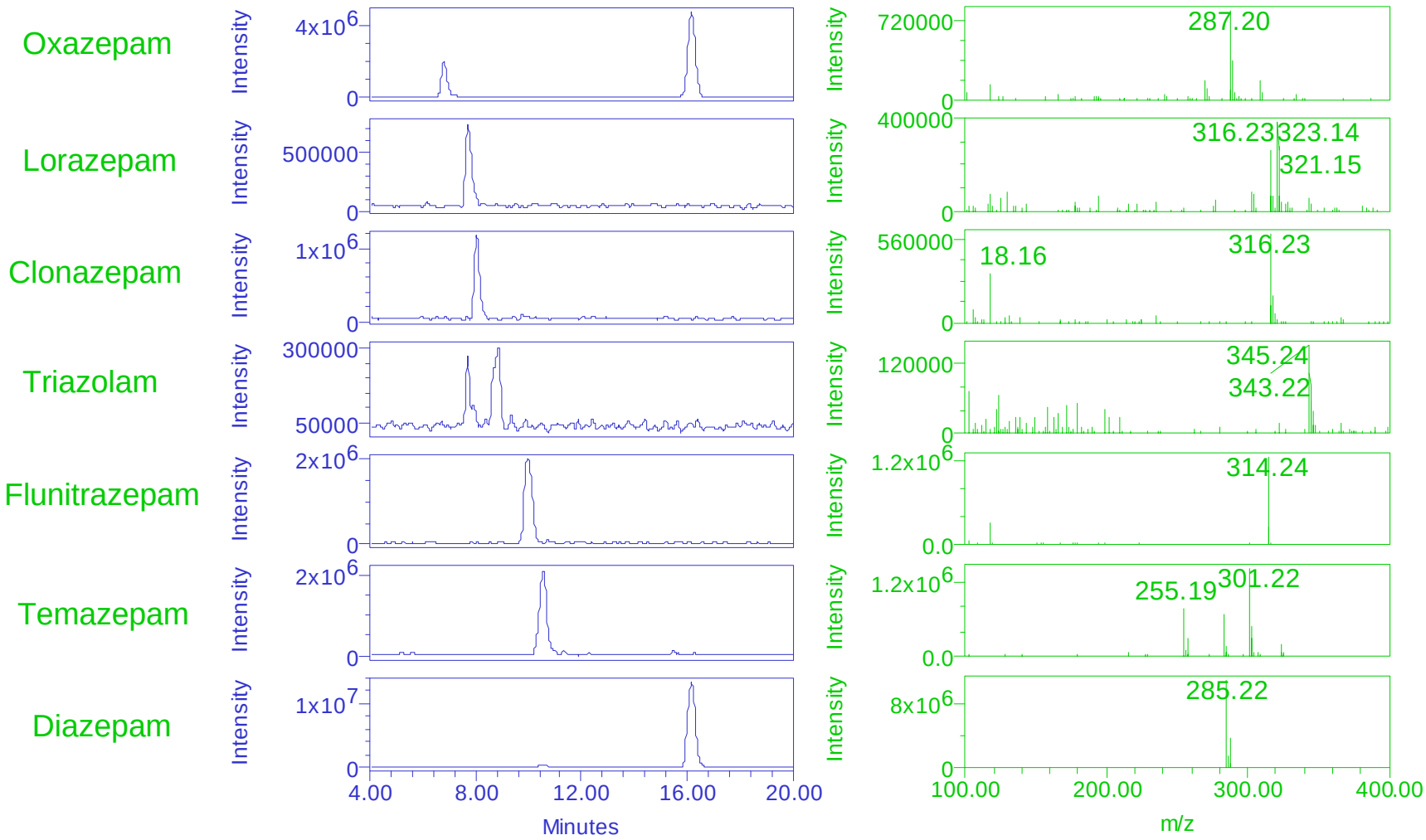


Serum Extract

25 ng on column
CV = 30 V

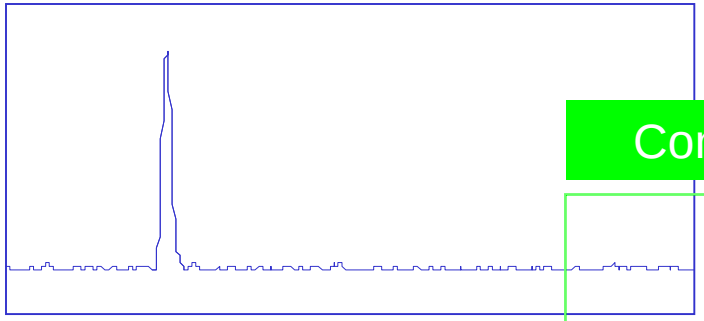
Extracted and Smoothed
Ion Chromatograms

Combined Mass Spectra

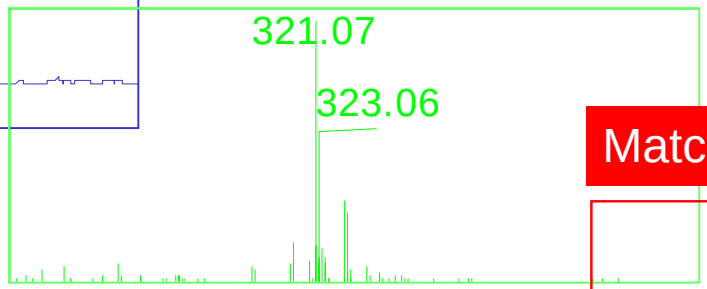


Identifying Sample Components

Extract Ion Chromatogram



Combine Mass Spectra



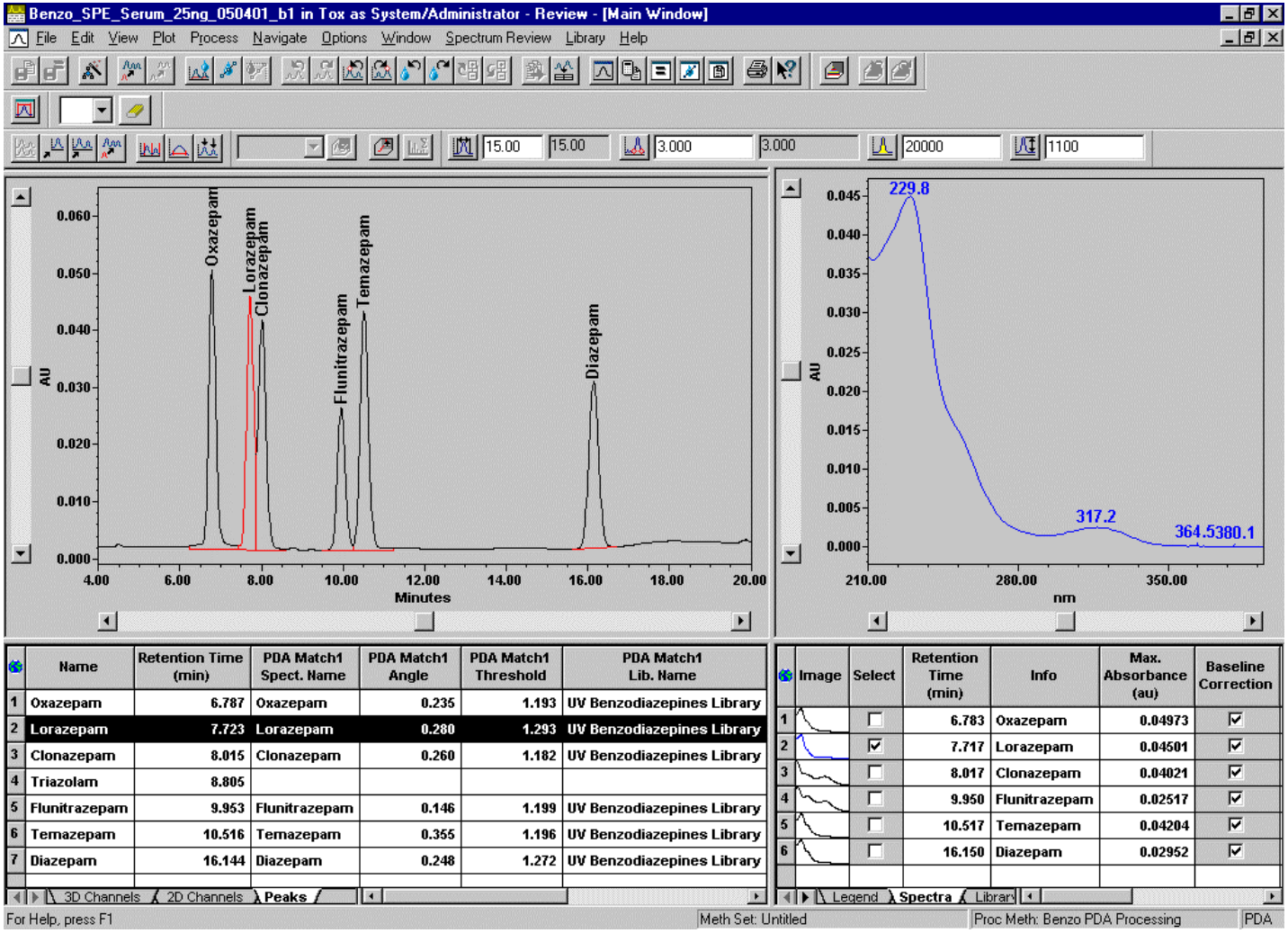
Match Against Library Spectra

Serum sample
25 ng on column
Cone voltage 30 V
Lorezepam

Processed Results from Serum Sample

Interactive analysis of processed PDA results in review window

25 ng on column



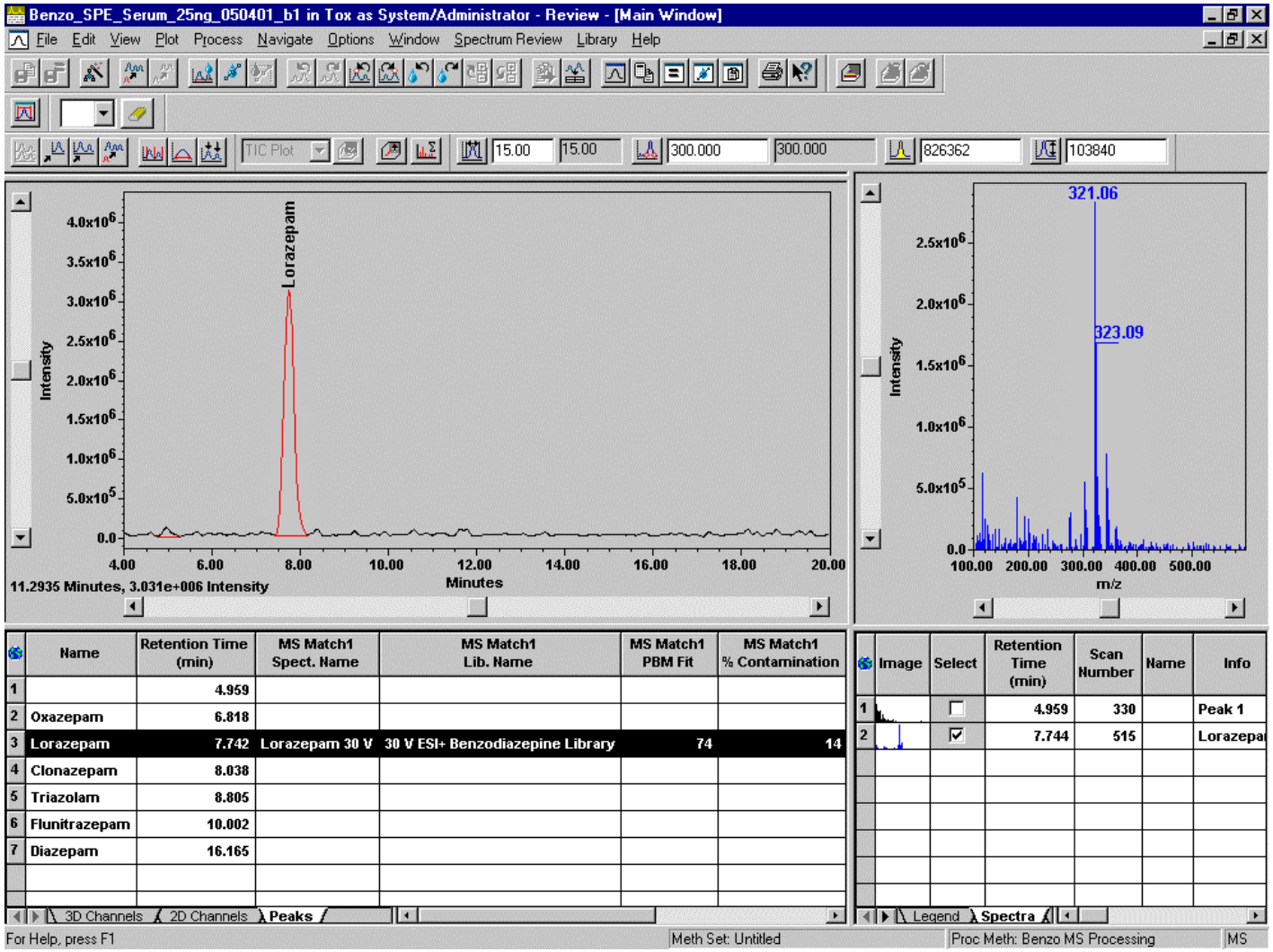
	Name	Retention Time (min)	PDA Match1 Spect. Name	PDA Match1 Angle	PDA Match1 Threshold	PDA Match1 Lib. Name
1	Oxazepam	6.787	Oxazepam	0.235	1.193	UV Benzodiazepines Library
2	Lorazepam	7.723	Lorazepam	0.280	1.293	UV Benzodiazepines Library
3	Clonazepam	8.015	Clonazepam	0.260	1.182	UV Benzodiazepines Library
4	Triazolam	8.805				
5	Flunitrazepam	9.953	Flunitrazepam	0.146	1.199	UV Benzodiazepines Library
6	Temazepam	10.516	Temazepam	0.355	1.196	UV Benzodiazepines Library
7	Diazepam	16.144	Diazepam	0.248	1.272	UV Benzodiazepines Library

	Image	Select	Retention Time (min)	Info	Max. Absorbance (au)	Baseline Correction
1		☐	6.783	Oxazepam	0.04973	☑
2		☑	7.717	Lorazepam	0.04501	☑
3		☐	8.017	Clonazepam	0.04021	☑
4		☐	9.950	Flunitrazepam	0.02517	☑
5		☐	10.517	Temazepam	0.04204	☑
6		☐	16.150	Diazepam	0.02952	☑

Processed Results from Serum Sample

Interactive analysis of processed MS results in review window

25 ng on column



	Name	Retention Time (min)	MS Match1 Spect. Name	MS Match1 Lib. Name	MS Match1 PBM Fit	MS Match1 % Contamination
1		4.959				
2	Oxazepam	6.818				
3	Lorazepam	7.742	Lorazepam 30 V	30 V ESI+ Benzodiazepine Library	74	14
4	Clonazepam	8.038				
5	Triazolam	8.805				
6	Flunitrazepam	10.002				
7	Diazepam	16.165				

	Image	Select	Retention Time (min)	Scan Number	Name	Info
1		☐	4.959	330		Peak 1
2		☑	7.744	515		Lorazepa

Search Results from Serum

25 ng on column	30 V	50 V	70 V	PDA	RT
Oxazepam	✓	✓	✓	✓	✓
Lorazepam	✓	✓	✓	✓	✓
Clonazepam	✓	✓	✓	✓	✓
Triazolam	✓†	✓†	x	x	✓
Flunitrazepam	✓‡	✓	✓	✓	✓
Temazepam	✓	✓	✓	✓	x*
Diazepam	✓	✓	✓	✓	✓

✓ Library match>60%
✓ Library match>20%
x Not found

† Library match against 50 V library
‡ Library match against 30 V library
* Manual inspection confirmed RT within reason for ID

Future Enhancements and Investigations

- Integrate EI-MS data with PDA and ESI-MS
- Further develop processing methods to improve MS library matches at lower concentrations
- Test robustness of libraries with time
- Investigate method transferability among instruments

Summary

- Multiple pieces of information can be generated in 1 sample run by collecting PDA and CID MS data
- Automated processing of data saves time and simplifies manual review of data
- Improved confidence in results
  - 5 characteristic features of a compound can be acquired and compared against standards
    - Retention time, UV spectra, and MS spectra at 3 cone voltages
- Easy to implement and relatively inexpensive
  - One software platform for acquisition and analysis
  - Single quadrupole methodology
    - Simple to set up
    - Less costly than MS/MS

Acknowledgements

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