Application Note

Forensic Toxicology

Agilent

Trusted Answers

Toxicological Drug Screening Using the LC Screener Tool with High-Resolution LC/Q-TOF

Figure 1. Revident LC/Q-TOF with 1290 Infinity II LC.

Authors

Cate Simmermaker,
Sierra D. Durham, Peter Stone,
and Lee Bertram
Agilent Technologies, Inc.

Abstract

This application note details a methodology for toxicological drug screening in complex biological matrices. The method was developed on the Agilent Revident liquid chromatography/quadrupole time-of-flight mass spectrometer (LC/Q-TOF MS) with the Agilent ChemVista spectral library manager and Agilent MassHunter Quantitative Analysis software, version 12.1. The LC Screener tool, which is embedded in MassHunter Quantitative Analysis software, was used to quickly review results of a data-independent acquisition (DIA) method for a wide range of target analytes over a typical large concentration range. This application note describes a complete screening workflow including sample preparation, suspect screening, and data analysis results for the screening of toxicological drugs in relevant biological matrices.

Introduction

Screening biological matrices for the presence of toxicological drugs by DIA offers the benefits of full screening. This screening method is not possible with targeted acquisition since it lacks the ability to retroactively analyze for the continuous emergence of new compounds of interest. Using DIA with applied collision energies furthers the level of information gathered allowing for the ability to differentiate coeluting isomers. The All Ions acquisition technique provides the analyst with full and unfettered access to the analytes of interest along with fragmentation information for identification confidence.

High-resolution LC/Q-TOF facilitates identification even further with an extended dynamic range, stable accurate mass, and isotopic fidelity. The DIA workflow in complex matrices with the new Revident LC/Q-TOF features key performance elements including a new detector, better mass accuracies, and increased dynamic range compared to previous generations of similar instruments. In addition, the use of ChemVista with LC/Q-TOF spectral libraries and databases can be combined with the LC Screener tool for routine drug analysis testing.

Experimental

Sample preparation

Two matrices, plasma and urine, were prepared by EMR–Lipid cartridge solid phase extraction and dilution, respectively. The plasma extract was reconstituted in a 60:40 methanol:water (MeOH:H_2O) solvent and the same solvent mixture was used for a 10:1 dilution of the urine.

The sample matrices were spiked with 32 scheduled drugs at eight concentration levels between 1 and 100 ng/mL, in addition to 16 heavy-labeled analytes spiked in at 50 ng/mL for use as internal standards. The samples were analyzed with reverse phase chromatography on an Agilent 1290 Infinity II LC and All Ions MS acquisition on a Revident LC/Q-TOF, including collision energies 0, 20, and 40 V. Two reference ions were used to ensure mass accuracy.

Equipment

Sample separation was performed using the 1290 Infinity II LC system, consisting of the following modules:

- Agilent 1290 Infinity II high-speed pump
- Agilent 1290 Infinity II multisampler with thermostat
- Agilent 1290 Infinity II multicolumn thermostat

The LC system was coupled to the Agilent Revident LC/Q-TOF mass spectrometer equipped with the Dual AJS ESI source. Agilent MassHunter Workstation software, version 12.0, was used for data acquisition. Agilent MassHunter Quantitative Analysis software, version 12.1, was used for the LC Screener tool and Agilent ChemVista library manager software, version 1.0, was used to curate a forensic toxicology library.

Methods

Liquid chromatography

Table 1. Agilent 1290 Infinity II LC method.

LC Conditions	
Parameter	**Value**
Column	Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)
Sampler Temperature	5 °C
Mobile Phase A	H_2O + 0.1% formic acid (FA) + 5 mM ammonium formate + 0.05 mM ammonium fluoride
Mobile Phase B	MeOH + 0.1% FA + 5 mM ammonium formate + 0.05 mM ammonium fluoride
Flow Rate	0.5 mL/min
Injection Volume	4 µL
Column Temperature	55 °C
Gradient Program	Time (min) %B 0.0 5 0.5 8 1.2 11 2.0 25 6.0 45 7.5 70 8.5 98 9.5 98 10.0 5

Mass spectrometry

Data were acquired on the Revident LC/Q-TOF (Figure 1), the new Agilent high-resolution instrument. The Revident facilitates identification even further with an extended dynamic range, stable accurate mass, and isotopic fidelity.

Table 2. LC/MS parameters.

Revident LC/Q-TOF	
Parameter	**Value**
Ion Source	Dual AJS ESI
Polarity	Positive
Gas Temperature	250 °C
Drying Gas Flow	11 L/min
Nebulizer Pressure	40 psi
Sheath Gas Temperature	350 °C
Sheath Gas Flow	12 L/min
Capillary Voltage	2,500 V
Nozzle Voltage	0 V
Collision Energies	0, 20, and 40 V

Results and discussion

Confident identifications: mass accuracy, stable area counts, and coeluting fragment ions

Independent of the matrix, compounds were measured with high mass accuracies, on average within ± 1 ppm and percent relative standard deviations (%RSDs) under 20% for good chromatographic precision. All compounds showed good linearity over all the acquired concentration levels, 1 to 100 ng/mL.

Table 3. List of R^2 values, average mass accuracies (MA), and area %RSDs (n = 4) over all detectable levels of plasma and urine.

	Plasma			Urine		
Compound	**R^2**	**Average MA (ppm)**	**Area %RSD**	**R^2**	**Average MA (ppm)**	**Area %RSD**
(±)-11-nor-9-Carboxy-Δ9-THC	0.992	−0.092	14.2	0.981	0.109	18.84
6-Acetylmorphine	0.998	0.211	1.52	0.999	0.512	11.15
Alprazolam	0.997	0.007	1.96	0.998	0.203	13.09
Amphetamine	0.996	0.352	6.8	0.994	0.862	18.69
Benzoylecgonine	0.995	0.188	1.42	0.997	0.368	11.04
Clonazepam	0.995	0.011	1.34	0.999	0.141	13.77
Cocaine	0.992	0.03	1.37	0.998	0.202	10.32
Codeine	0.998	0.124	2.43	0.998	0.245	13.95
Diacetylmorphine	0.999	−0.045	1.85	0.997	−0.062	14.99
Diazepam	0.999	0.103	1.44	0.998	0.369	11.16
Hydrocodone	0.999	0.101	2.56	0.998	0.146	13.42
Hydromorphone	0.996	0.331	2.23	0.996	0.451	17.54
Lorazepam	0.998	0.225	2.73	0.998	0.597	14.16
MDA	0.998	0.398	6.39	0.991	0.605	9.38
MDEA	0.999	0.137	1.51	0.999	0.552	11.29
MDMA	0.999	0.045	3.91	0.999	0.308	14.34
Meperidine	0.999	0.132	0.68	0.991	0.552	9.79
Methadone	0.998	0.145	1.67	0.997	0.342	10.48
Methamphetamine	0.999	0.336	1.82	0.999	0.595	9.43
Morphine	0.994	0.195	1.45	0.997	0.536	15.42
Nitrazepam	0.998	0.073	1.25	0.991	0.325	13.33
Oxazepam	0.997	0.062	1.73	0.999	0.374	13.15
Oxycodone	0.999	−0.002	1.17	0.999	0.204	12.19
Oxymorphone	0.995	0.227	1.31	0.990	0.477	16.74
Phencyclidine	0.999	0.034	1.92	0.999	0.465	11.08
Phentermine	0.999	0.335	1.82	0.998	0.658	10.94
Proadifen	0.999	0.109	1.84	0.997	0.211	11.54
Strychnine	0.993	−0.026	1.66	0.998	0.101	11.28
Temazepam	0.992	−0.321	1.97	0.999	0.107	12.72
Trazodone	0.999	−0.004	1.47	0.997	−0.019	13.08
Verapamil	0.997	0.028	1.46	0.996	−0.266	13.21
Δ9-THC	0.996	0.278	11.4	0.996	0.271	18.07

Typical screening parameters were set to mass accuracies of < 5 ppm, retention time alignment, coelution of fragment ions, signal-to-noise (S/N) > 3, and mass match score. Using the screening parameters, MassHunter Quantitative Analysis 12.1 and the LC Screener tool assign a designation of detected, questionable, or not detected based on alignment that is easy to review. The mass accuracy measurement of all individual analytes is shown in Figure 2, displaying the lowest analyzed concentration (1 ng/mL) on the left to the greatest analyzed concentration (100 ng/mL) on the right, including over 1,900 data points.

This designation of detected or undetected status by the LC Screener tool is accompanied by clear chromatograms displaying coeluting fragment ions, as shown in Figure 3. The fragment ions are imported from available spectra in curated libraries with retention times developed from neat standards and are available in the Agilent LC/Q-TOF Applied Markets library in ChemVista.

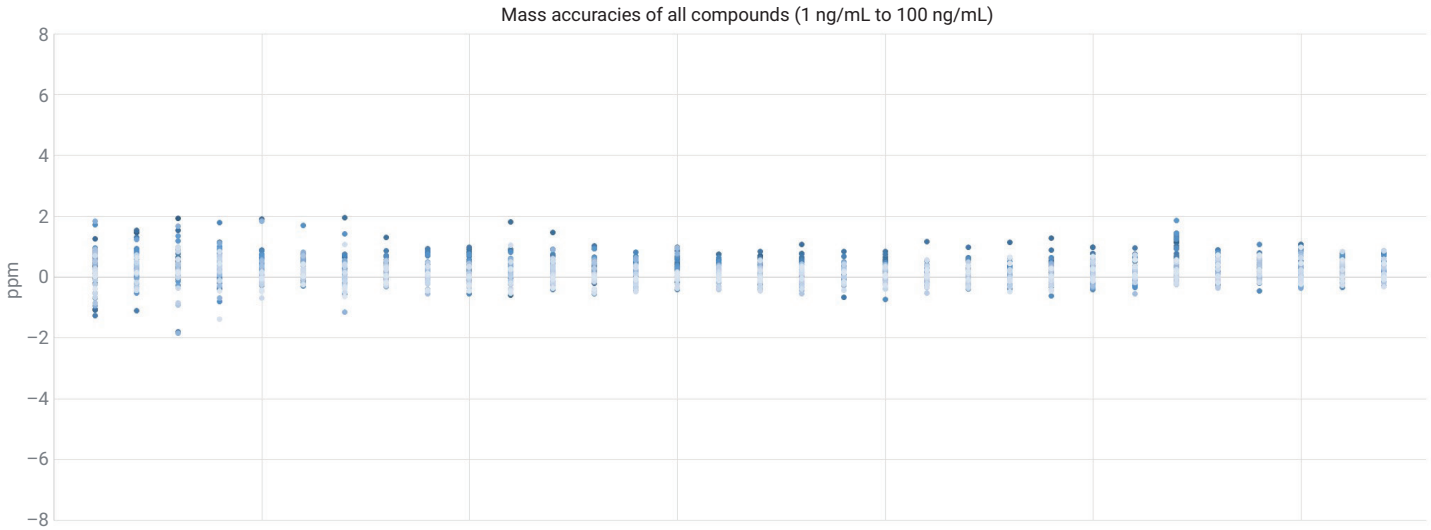

Figure 2. Mass accuracy measurements of all analytes from 1 ng/mL (left) to 100 ng/mL (right).

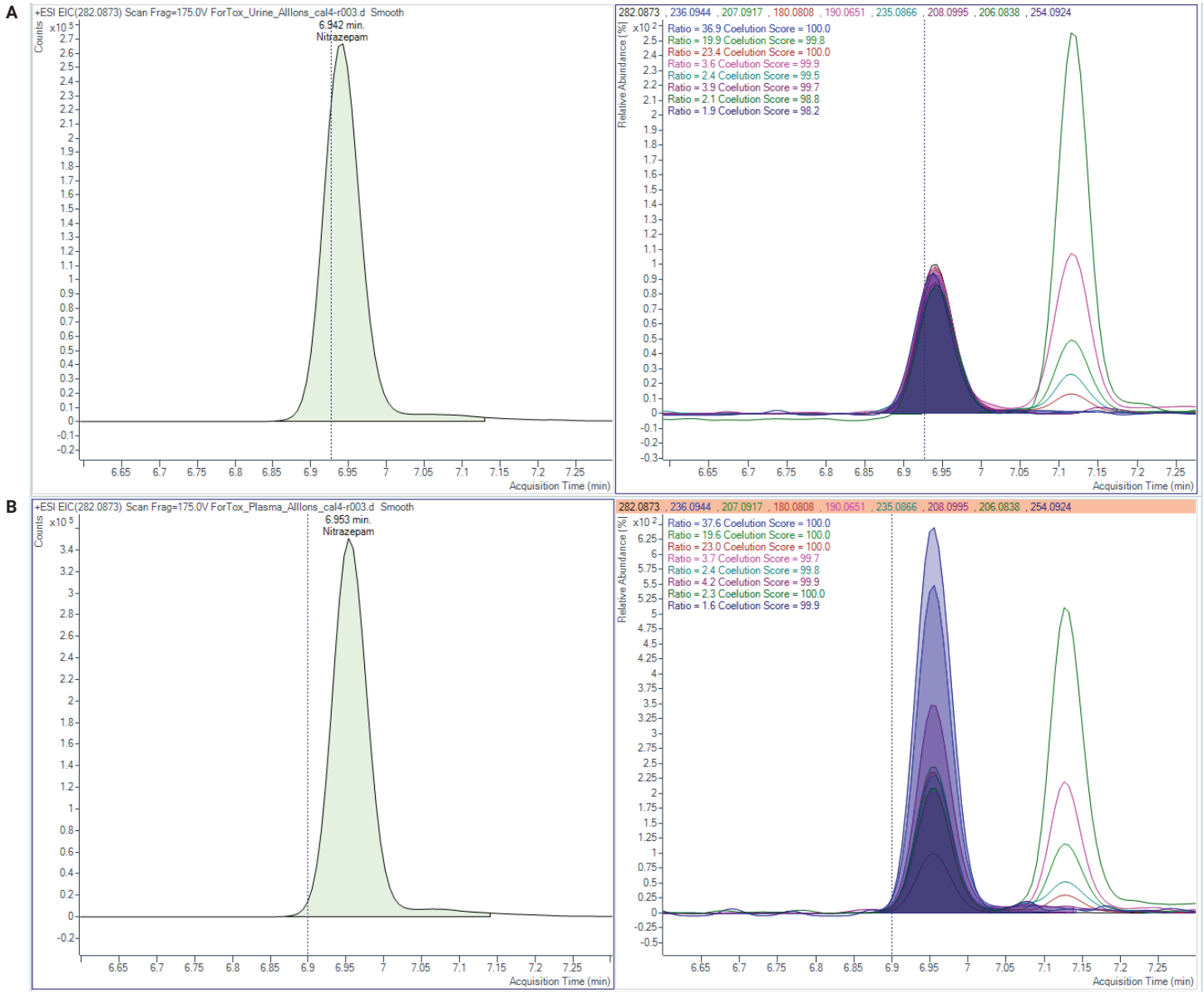

Figure 3. Quantifier (left) and qualifier ions (right) for nitrazepam (20 ng/mL) in urine (A) and plasma (B).

Spectral library and database management using ChemVista

In this experiment, ChemVista was used to build out the spectral libraries for the targets of interest by importing third party spectral information. The imported spectra provided effective fragment ions for comparison with an All Ions data file. The structure of ChemVista allows easy management of existing libraries along with the simple workflow for inclusion of new compounds as they are required. An example of the compound information available in ChemVista can been seen in Figure 4.

The ChemVista library manager software provides streamlined library management and includes extensive, curated, high-resolution spectral libraries. Additionally, ChemVista can import essential compound, structure, and spectral information from open-access libraries, such as MassBank of North America and the EPA CompTox Chemicals Dashboard. ChemVista integrates spectral and retention time (and/or index) information collected under different MS conditions and can support multiple retention times to reflect various chromatographic separation methods. The compound-centric data model is based on a cheminformatic underpinning, ensuring compounds are included without duplication.

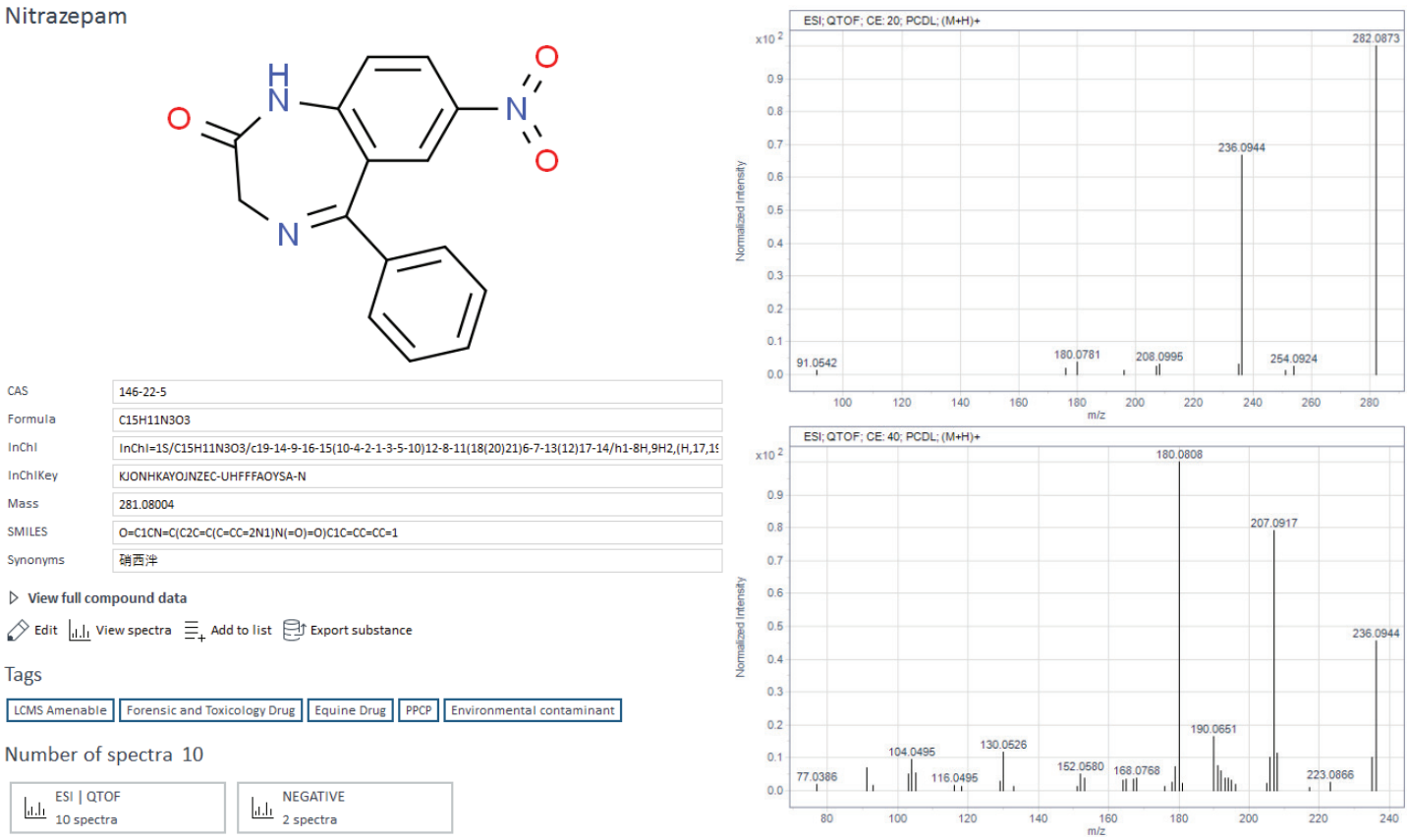

Figure 4. Agilent ChemVista library information example for nitrazepam, which includes chemical identifiers, tags, and spectra.

Efficient library management is accomplished through list organization, intuitive searching, and filtering options to facilitate identification workflows within the MassHunter data analysis applications, such as MassHunter Quantitative Analysis 12.1, which includes the LC Screener tool.

Individual compounds are labeled with chemical identifiers and accompanied by tags for quick and effective searching and sorting, as seen with the drug metabolite nitrazepam in Figure 4. In addition to the compounds in this screening, the the Applied Markets library contains over 9,500 forensic and toxicology drugs (4,500 with spectra) and includes compounds such as fentanyl derivatives and novel psychoactive substances (NPS). ChemVista further supports easy addition of NPS through multiple import formats and simple creation and editing of chemical structure information to jump-start untargeted screening for putative identification. For more detail see the Agilent ChemVista Library Manager technical overview.

Simplified untargeted screening data analysis workflow with the LC screener tool

Easy access to the results of large screening methods assists in the review loading and time of analysis. The LC Screener tool, a function embedded into MassHunter Quantitative Analysis, aids in the review of these screening experiments by using outlier parameters to review data and assign a designation of identified, questionable, or not detected. The screening outlier parameters, as shown in Figure 5, include retention time difference, mass match score, mass accuracy, minimum S/N, and a verified ion cutoff. This cutoff can be related to fragments or isotopes depending on the preferred acquisition mode. A tally of the designations associated with all the screened compounds is kept on the top right of the LC Screener page and filter settings are on the top left.

Details related to the setup of the LC Screener tool can be found in the detailed application notes cited in the references.

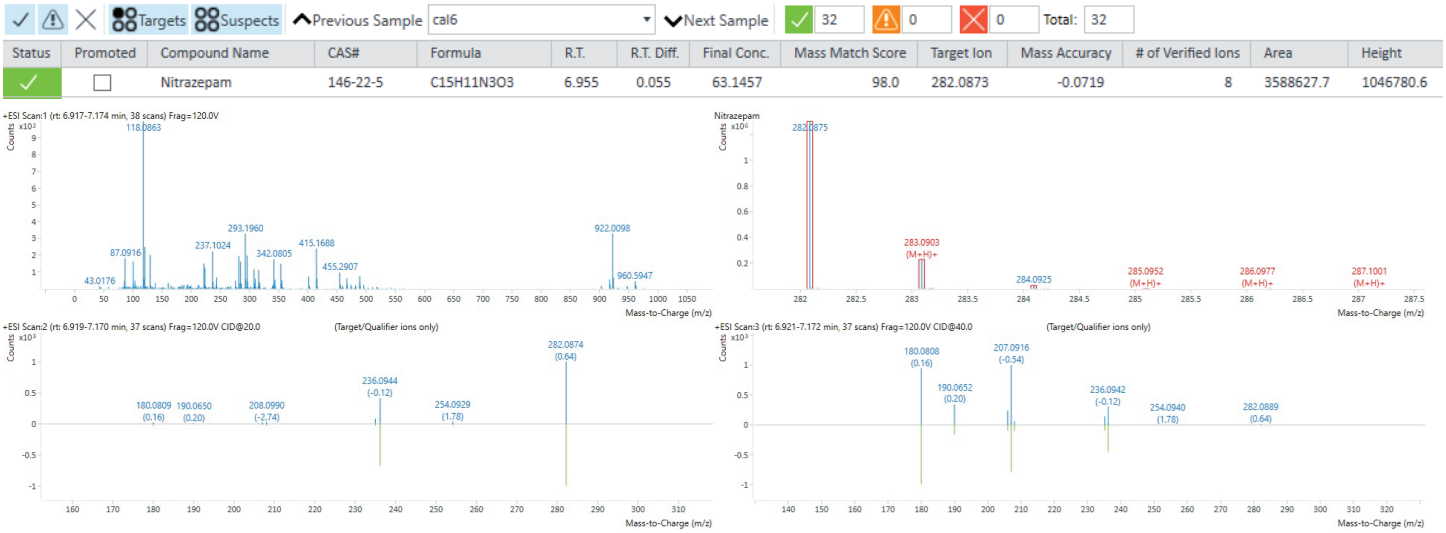

Figure 5. LC Screener tool user interface, highlighting nitrazepam, including spectra at 0, 20, and 40 V along with molecular ion and isotope abundance experimental information compared to theoretical.

Conclusion

Using the latest software releases from Agilent including MassHunter Acquisition software, ChemVista library manager, and MassHunter Quantitative Analysis software combined with the Agilent Revident LC/Q-TOF delivers excellence in mass accuracy. With these tools, untargeted screening of drugs in multiple matrices can be done quickly and confidently. This application illustrates high mass accuracy and isotopic fidelity for the toxicology analytes in this study. These results are illustrated across a high concentration range together with low chromatographic %RSDs provided from the Revident LC/Q-TOF. The use of a curated custom forensic toxicology library in ChemVista and use of LC Screener tool provided by MassHunter Quantitative Analysis software achieves a simple and effective screening workflow for forensic and toxicological compounds in complex matrices.

References

1. Zhao, L. Quantitative Determination of Drugs of Abuse in Human Whole Blood by LC/MS/MS Using Agilent Captiva EMR—Lipid Cleanup, *Agilent Technologies application note*, publication number 5991-9251EN, **2018**.
2. Yannell, K. E.; Gomez, M. Drug Screening in Whole Blood Using the Agilent 6546 LC/Q-TOF and the LC Screener Tool with Automated Sample Preparation, *Agilent Technologies application note*, publication number 5994-1744EN, **2020**.

www.agilent.com

RA45559.6458449074

Printed in the USA, October 28, 2024
5994-7850EN

Agilent
Trusted Answers