Highly sensitive MS/MS detection for confident identification of potent novel synthetic opioids and their metabolites

HRMS analysis of discarded authentic postmortem case samples using the SCIEX ZenoTOF 7600 system, powered by SCIEX OS software

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The introduction of highly potent novel synthetic opioids (NSO) to the illicit drug market has been a major driver for the recent rise in the number of accidental drug overdoses. NSO are a class of novel psychoactive substances (NPS) that are commonly used as adulterants in heroin and counterfeit preparations to mimic the effects of controlled opioids. These substances vary greatly in potency and purity and thus often require only a small amount to cause acute intoxications. Their increasing occurrence in combined opioid drug toxicity cases, resulting in accidental and fatal drug overdoses, continues to create a major challenge for public health officials.

Traditionally, screening for ultra-potent substances was performed using targeted workflows, such as multiple reaction monitoring (MRM) using triple quadrupole mass spectrometers, because of the higher selectivity and sensitivity performance. However, the continuous emergence of NPS on the recreational drug market is creating an additional challenge for drug tracking agencies and laboratories to meet. High-resolution mass spectrometry has provided forensic toxicology laboratories with a unique tool for the untargeted detection and identification of these new emerging substances, with little or no method optimization necessary. In addition, accurate mass instruments are affording additional levels of certainty by reliably obtaining comprehensive MS/MS spectral fragment information that can be used for identification, confirmation, and/or library matching.

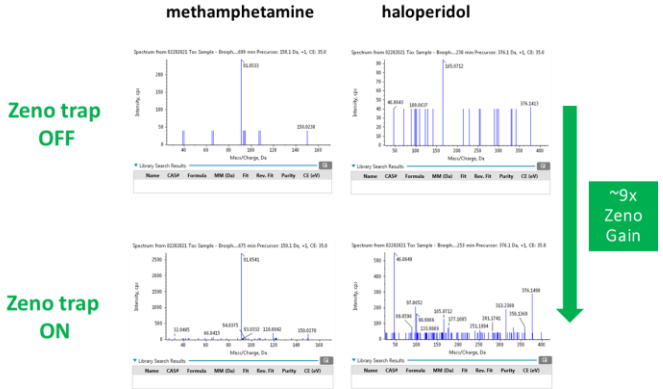


Figure 1: TOF MS/MS sensitivity gains using Zeno IDA for representative analytes. An average of ~9X gain in TOF MS/MS sensitivity was observed across all analytes identified in this study.

In this technical note, a highly sensitive method for the detection and identification of potent NSO in human whole blood is described. The technological enhancements of the ZenoTOF 7600 system[1] provide a high degree of sensitivity, selectivity and confidence for MS/MS experiments. They enable accurate and reliable detection of potent substances in poly-drug, authentic, case samples at trace levels that were not previously achievable.

Key features of Zeno IDA for untargeted detection of low level NSO in blood samples

- Zeno trap provides ≥90% duty cycle across the entire mass range for MS/MS acquisition
- Improved duty cycle leads to an MS/MS sensitivity increase, resulting in higher numbers of detections, improved spectral library matching and increased confidence in identification
- MS/MS sensitivity improvements of ~9X, on average, across all MS/MS fragments for the positively identified substances
- Increased MS/MS sensitivity leads to confident detection of low level NPS, metabolites and other potent drugs in discarded authentic postmortem case samples, providing the necessary evidence to support medicolegal death investigations

Experimental details

Target analytes: An NSO panel including 3 newly emerging non-fentanyl opioids (brorphine, isotonitazene, metonitazene), one metabolite (4'-hydroxy nitazene) and two halogenated fentanyl analogs (*para*-fluorofentanyl and *para*-chlorofentanyl) was selected for method development. A 1 µg/mL standard mixture containing the 6 target analytes and a 1 ng/mL fentanyl-D5 internal standard solution were prepared in water.

Calibrator preparation: The 1 µg/mL standard mixture containing the 6 target analytes was used to fortify 500 µL of human whole blood. This freshly spiked whole blood mixture was used to prepare a series of 9 calibrator solutions covering concentrations ranging from 10 pg/mL to 100 ng/mL.

Sample preparation: NSO were extracted from human whole blood using a liquid-liquid extraction (LLE) procedure summarized in Figure 2.

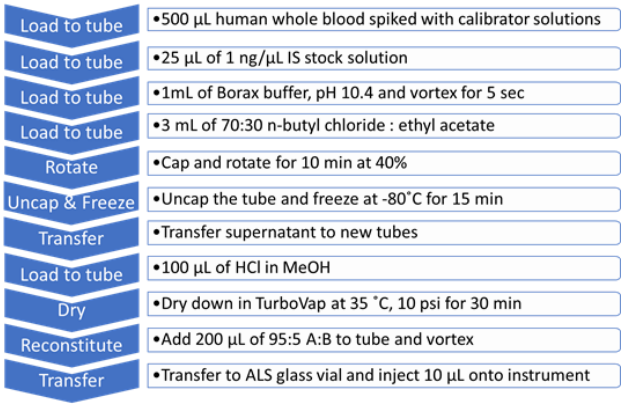


Figure 2. Liquid-liquid extraction (LLE) procedure for human whole blood samples. A 10-step extraction protocol was used for selectively extracting drugs from human whole blood samples for analysis with the ZenoTOF 7600 system.

Liquid chromatography: HPLC separation was performed on an ExionLC system using a Phenomenex Kinetex C18 column (50 × 3.0 mm, 2.6 µm, 00B-4462-Y0). Mobile phase A (MPA) and mobile phase B (MPB) were ammonium formate (pH 5) and formic acid in methanol and acetonitrile, respectively. The flow rate was 0.4 mL/min with a total LC runtime of 15.5 minutes. The injection volume was 10 µL.

Mass spectrometry: MS and MS/MS data were collected for each sample using Zeno IDA for optimal sensitivity on the ZenoTOF 7600 system. Data acquisition consisted of a TOF MS scan to collect accurate mass precursor ions from 100 to 700 Da, followed by a TOF MS/MS full scan ranging from 25 to 700 Da to ensure all fragments were captured for identification using a maximum of 16 candidate ions. Data was acquired using SCIEX OS software 2.0.1.

Data analysis: Data was processed using SCIEX OS software 2.0.1. Detection and integration of the peaks from the background was accomplished using the MQ4 algorithm in the Analytics module of the software where quantitative and qualitative analyses were performed. Positive analyte identification was accomplished based on confidence criteria as previously described.[2] The four main confidence criteria used include mass error (M), retention time (R), isotope ratio difference (I), and library score (L). An in-house library was used to perform spectral library matching and identification of the drugs present in the discarded authentic postmortem case samples.

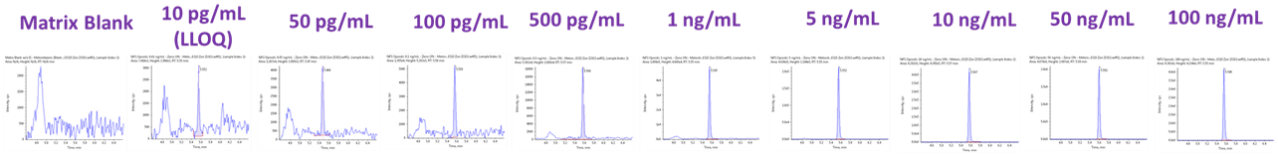
Optimized IDA method leads to accurate and reliable drug quantification

Information dependent acquisition (IDA) is a non-targeted data dependent acquisition technique that provides high confidence in compound identification by generating high-resolution, accurate mass spectra in both MS and MS/MS modes for spectral library matching or for structural elucidation purposes. Accurate quantification can also be performed simultaneously using the accurate mass of precursor ions from the TOF MS experiment.

A series of 9 calibrator solutions were prepared by spiking control human whole blood samples with the 6 targeted analytes at final concentrations ranging from 10 pg/mL to 100 ng/mL. The series of calibrator solutions were injected to evaluate the quantitative performance of the system and its ability to accurately measure low level analytes with a high level of precision and accuracy in TOF MS mode. Each calibrator was injected in triplicate.

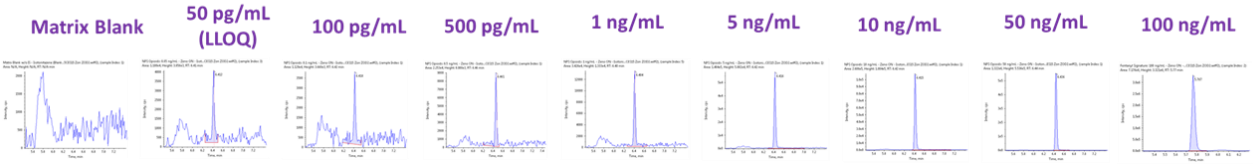
Figure 3 shows representative extracted ion chromatograms (XICs) for A) metonitazene and B) isotonitazene, two highly potent NSO that have been linked to accidental drug overdoses at low concentrations. The series of XIC displays shows the resulting signal for a blank injection (left) and for concentrations ranging from 10 pg/mL (LLOQ) to 100 ng/mL for metonitazene and from 50 pg/mL (LLOQ) to 100 ng/mL for isotonitazene, respectively. Figure 3 also displays the statistical results from the peak area integration of A) metonitazene and B) isotonitazene. Excellent precision and accuracy were observed across the series of calibrators, proving the robustness of the assay. Full quantification, including detection and integration of the peaks and area, concentration and quantitative performance value calculations (precision and accuracy) was automatically performed in Analytics in SCIEX OS software. The software is designed for quick, intuitive and streamlined data processing with accurate and reliable results.

A Metonitazene



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	Metonitazene	0.01	3 of 3	9.875e-3	1.477e-3	14.95	98.75	9.474e-3	8.641e-3	1.151e-2
2	Metonitazene	0.05	3 of 3	5.166e-2	1.685e-3	3.26	103.33	5.079e-2	5.059e-2	5.361e-2
3	Metonitazene	0.10	3 of 3	9.053e-2	4.580e-3	5.06	90.53	8.525e-2	9.292e-2	9.342e-2
4	Metonitazene	0.50	3 of 3	5.183e-1	2.291e-2	4.42	103.66	5.432e-1	4.982e-1	5.134e-1
5	Metonitazene	1.00	3 of 3	1.023e0	1.640e-2	1.60	102.34	1.039e0	1.026e0	1.006e0
6	Metonitazene	5.00	3 of 3	5.091e0	5.074e-2	1.00	101.82	5.086e0	5.043e0	5.144e0
7	Metonitazene	10.00	3 of 3	9.814e0	3.087e-1	3.15	98.14	1.011e1	9.492e0	9.842e0
8	Metonitazene	50.00	3 of 3	5.137e1	8.919e-1	1.74	102.73	5.219e1	5.042e1	5.149e1
9	Metonitazene	100.00	3 of 3	9.870e1	2.265e0	2.29	98.70	9.996e1	1.000e2	9.608e1

B Isotonitazene



Row	Component Name	Actual Concentration	Num. Values	Mean	Standard Deviation	Percent CV	Accuracy	Value #1	Value #2	Value #3
1	Isotonitazene	0.01	0 of 3	N/A	N/A	N/A	N/A	~~1.706e-1~~	~~9.531e-1~~	N/A
2	Isotonitazene	0.05	3 of 3	5.563e-2	5.110e-3	9.19	111.27	5.854e-2	4.973e-2	5.863e-2
3	Isotonitazene	0.10	3 of 3	9.682e-2	1.776e-3	1.83	96.82	9.478e-2	9.801e-2	9.767e-2
4	Isotonitazene	0.50	3 of 3	5.135e-1	1.879e-2	3.66	102.70	4.994e-1	5.062e-1	5.348e-1
5	Isotonitazene	1.00	3 of 3	9.619e-1	4.868e-2	5.06	96.19	1.011e0	9.601e-1	9.141e-1
6	Isotonitazene	5.00	3 of 3	4.974e0	1.625e-1	3.27	99.47	5.091e0	5.042e0	4.788e0
7	Isotonitazene	10.00	3 of 3	9.240e0	2.412e-1	2.61	92.40	9.289e0	8.977e0	9.452e0
8	Isotonitazene	50.00	3 of 3	5.035e1	5.170e-1	1.03	100.70	5.091e1	5.025e1	4.989e1
9	Isotonitazene	100.00	3 of 3	1.005e2	7.097e-1	0.71	100.46	1.013e2	1.002e2	9.992e1

Figure 3. Extracted ion chromatogram (XIC) traces and statistical results for A) metonitazene and B) isotonitazene, two potent NSO targeted in this study. XIC traces and resulting statistics panes from 10 pg/mL (LLOQ) to 100 ng/mL for: A) metonitazene and from 50 pg/mL (LLOQ) to 100 ng/mL for B) isotonitazene, respectively. Both NSO showed excellent accuracy and precision across the calibration levels, proving the overall robustness of the assay.

XIC area values resulting from the TOF MS experiment were used to generate regression plots for each of the 6 targeted analytes. Figure 4 shows the resulting calibration curves which demonstrate excellent linearity across the concentration ranges analyzed. They were calculated with R^2 values observed to be greater than 0.99 for all 6 targeted NSO.

Table 1 lists the name, the calibration range, linear correlation value (R^2), and LLOQ, as well as the accuracy and precision reported at the LLOQ for each of the 6 target analytes used in this panel. These values demonstrate the quantitative performance of the ZenoTOF 7600 system in TOF MS mode.

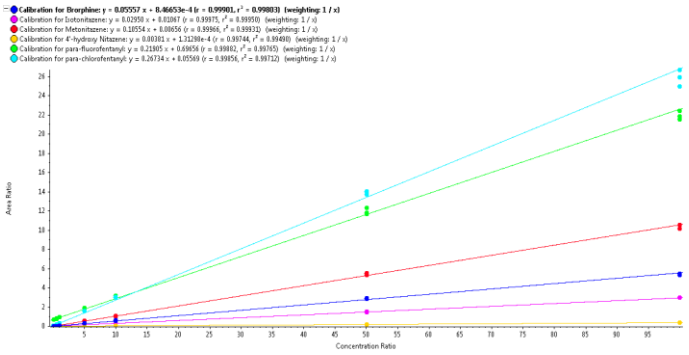


Figure 4. Excellent linearity for the 6 targeted NSO. Calibration curves resulting from the series of 9 calibrators extracted from human whole blood at concentrations ranging from 10 pg/mL to 100 ng/mL. R^2 values greater than 0.99 were observed for the 6 targeted analytes.

Table 1. Statistical results for the 6 targeted drugs. The table includes calibration range, linear correlation coefficient (R^2 Value), and LLOQ, as well as the accuracy and precision at the LLOQ for each of the 6 targeted drugs.

Compound	Calibration Range (ng/mL)	Linear Correlation (R2)	LLOQ (ng/mL)	Accuracy at LLOQ (%)	Precision at LLOQ (%)
Brorphine	*0.05 - 100*	*0.99803*	*0.05*	*88.95*	*8.47*
Isotonitazene	*0.05 - 100*	*0.99950*	*0.05*	*111.27*	*9.19*
Metonitazene	*0.01 - 100*	*0.99931*	*0.01*	*98.75*	*14.95*
4-Hydroxy Nitazene	*0.1 - 100*	*0.99490*	*0.1*	*103.58*	*7.52*
para-Fluorofentanyl	*0.5 - 100*	*0.99765*	*0.5*	*85.75*	*2.23*
para-Chlorofentanyl	*0.01 - 100*	*0.99712*	*0.01*	*88.24*	*8.17*

Zeno trap technology leads to MS/MS sensitivity gains

QTOF mass spectrometers commonly make use of an orthogonal TOF geometry which has been shown to maximize MS and MS/MS resolution and mass accuracy for an entire spectrum, but results in a significant loss of ions through this region of the MS (only 5-20% duty cycle).[1] To overcome this limitation, a Zeno trap was added at the end of the collision cell on the ZenoTOF 7600 system, which increases the duty cycle in the orthogonal injection region of the MS to ≥90% across the entire mass range. Therefore, the technological enhancements on the ZenoTOF 7600 system significantly increase MS/MS sensitivity which results in improved MS/MS spectral quality at low analyte concentration. This improvement ultimately yields improved MS/MS spectral library matching which provides greater confidence in analyte identification.

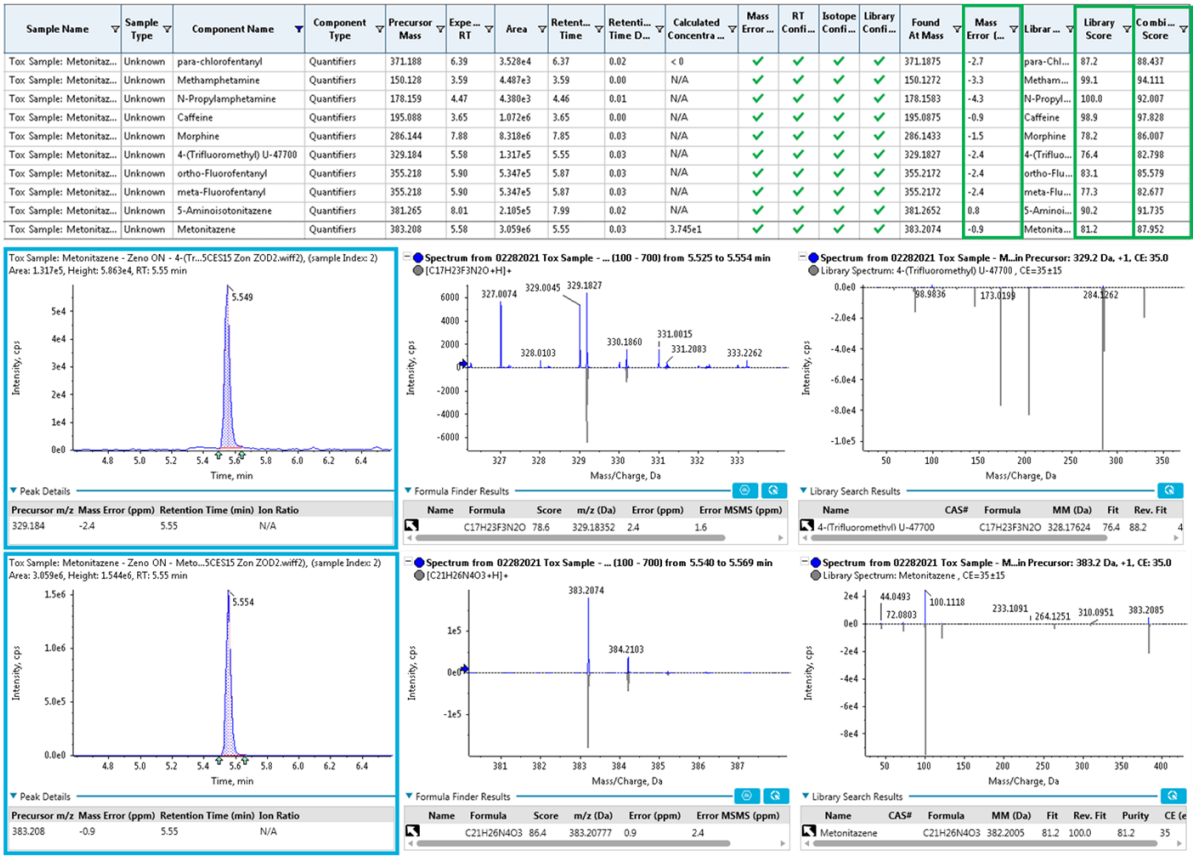
Zeno MS/MS increases confident identifications of low drug levels in authentic postmortem case samples

The MS/MS sensitivity improvements resulting from the use of the Zeno trap on the ZenoTOF 7600 system was investigated by analyzing discarded authentic postmortem case samples from subjects suspected of NSO ingestion resulting in accidental overdoses. These biological specimens were prepared using the aforementioned LLE procedure. Data were acquired on the ZenoTOF 7600 system with both the Zeno trap on and off for each sample and the results were compared to assess the impact of the MS/MS sensitivity gains. The concentrations of the targeted NSO detected in the discarded authentic postmortem case samples were calculated automatically in SCIEX OS software using the calibration curves generated for each of the 6 target analytes. Each case sample was run in triplicate.

Case study 1

Figure 5 (top) shows the results table from the analysis of discarded authentic postmortem case sample #1, using Zeno IDA, where 10 analytes were successfully identified. Figure 5 (bottom) also displays the XIC, TOF MS and TOF MS/MS spectra of two representative drugs positively identified in the sample: methamphetamine and 4-(Trifluoromethyl) U-47700, a potent synthetic opioid that has been reported to cause opioid-like effects similar to heroin and fentanyl. The results table shows the successful detection of two of the targeted NSO: *para*-chlorofentanyl and metonitazene, as well as other non-targeted NPS such as 4-(Trifluoromethyl) U-47700 and fluorofentanyl (the para and meta isomers were not resolved chromatographically). The presence of fentanyl analogs (*para*-chlorofentanyl and *para-/meta*- fluorofentanyl) and the potent synthetic opioid 4-(Trifluoromethyl) U-47700 suggest that the subject ingested a preparation originating from the illicit drug market. The presence of multiple potent NSO could support the case of combined opioid drug toxicity leading to death. Positive identification determination was accomplished using the four confidence criteria and sorted out using the traffic light system. The mass errors (ranging from -4.3 to 0.8 ppm), the mass spectra library scores (ranging from 76 to 100%) and the combined scores (ranging from 82.677 and 97.828%) provided excellent measures of the confident identification of the ten compounds in the discarded postmortem sample #1.

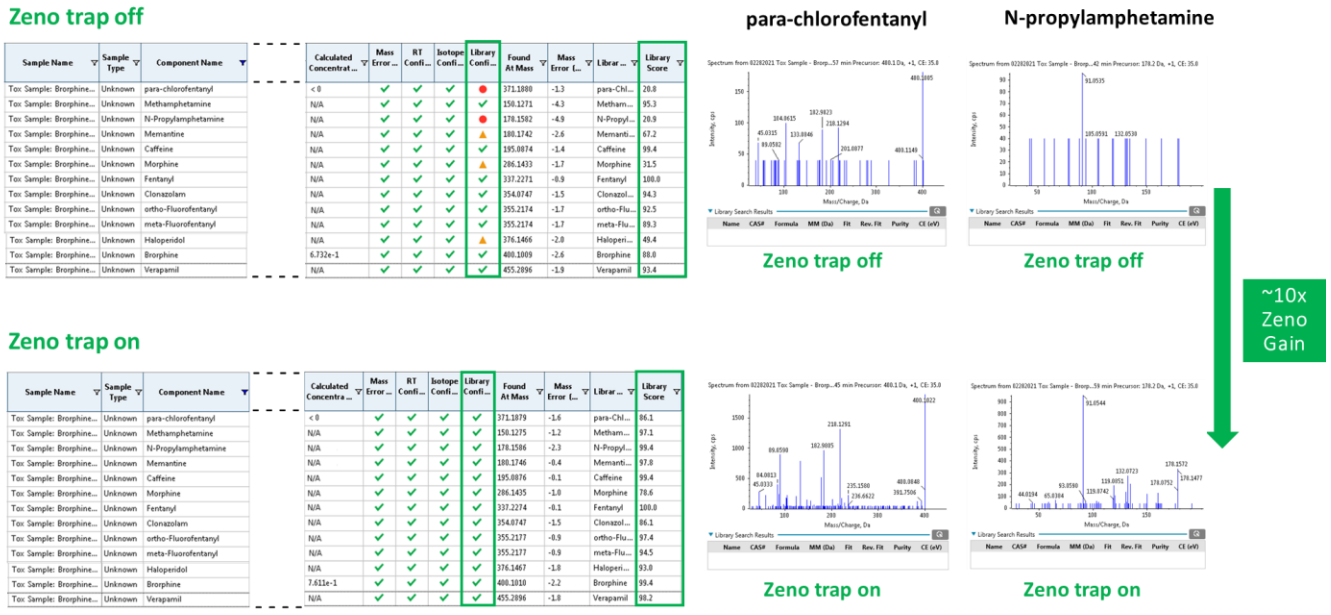
Discarded authentic postmortem case sample # 1



Sample Name	Sample Type	Component Name	Component Type	Precursor Mass	Expe... RT	Area	Retent... Time	Retenti... Time D...	Calculated Concentra ...	Mass Error ...	RT Confi...	Isotope Confi...	Library Confi...	Found At Mass	Mass Error (...	Librar ...	Library Score	Combi... Score
Tox Sample: Metonitaz...	Unknown	para-chlorofentanyl	Quantifiers	371.188	6.39	3.528e4	6.37	0.02	< 0	✓	✓	✓	✓	371.1875	-2.7	para-Chl...	87.2	88.437
Tox Sample: Metonitaz...	Unknown	Methamphetamine	Quantifiers	150.128	3.59	4.487e3	3.59	0.00	N/A	✓	✓	✓	✓	150.1272	-3.3	Metham...	99.1	94.111
Tox Sample: Metonitaz...	Unknown	N-Propylamphetamine	Quantifiers	178.159	4.47	4.380e3	4.46	0.01	N/A	✓	✓	✓	✓	178.1583	-4.3	N-Propyl...	100.0	92.007
Tox Sample: Metonitaz...	Unknown	Caffeine	Quantifiers	195.088	3.65	1.072e6	3.65	0.00	N/A	✓	✓	✓	✓	195.0875	-0.9	Caffeine	98.9	97.828
Tox Sample: Metonitaz...	Unknown	Morphine	Quantifiers	286.144	7.88	8.318e6	7.85	0.03	N/A	✓	✓	✓	✓	286.1433	-1.5	Morphine	78.2	86.007
Tox Sample: Metonitaz...	Unknown	4-(Trifluoromethyl) U-47700	Quantifiers	329.184	5.58	1.317e5	5.55	0.03	N/A	✓	✓	✓	✓	329.1827	-2.4	4-(Triflu...	76.4	82.798
Tox Sample: Metonitaz...	Unknown	ortho-Fluorofentanyl	Quantifiers	355.218	5.90	5.347e5	5.87	0.03	N/A	✓	✓	✓	✓	355.2172	-2.4	ortho-Flu...	83.1	85.579
Tox Sample: Metonitaz...	Unknown	meta-Fluorofentanyl	Quantifiers	355.218	5.90	5.347e5	5.87	0.03	N/A	✓	✓	✓	✓	355.2172	-2.4	meta-Flu...	77.3	82.677
Tox Sample: Metonitaz...	Unknown	5-Aminoisotonitazene	Quantifiers	381.265	8.01	2.105e5	7.99	0.02	N/A	✓	✓	✓	✓	381.2652	0.8	5-Aminoi...	90.2	91.735
Tox Sample: Metonitaz...	Unknown	Metonitazene	Quantifiers	383.208	5.58	3.059e6	5.55	0.03	3.745e1	✓	✓	✓	✓	383.2074	-0.9	Metonita...	81.2	87.952

Figure 5. Results from authentic postmortem case study #1. (Top) Results table in SCIEX OS software showing the analytes positively identified in postmortem case sample #1 along with mass error, library score and combined score using the confidence criteria. (Bottom) XICs, TOF MS and TOF MS/MS spectra collected provide detailed and confident identification of two of the positively identified analytes: methamphetamine and 4-(Trifluoromethyl) U-47700.

Discarded authentic postmortem case sample # 2



Sample Name	Sample Type	Component Name	Calculated Concentra ...	Mass Error ...	RT Confi...	Isotope Confi...	Library Confi...	Found At Mass	Mass Error (...	Librar ...	Library Score
Tox Sample: Brorphine...	Unknown	para-chlorofentanyl	< 0	✓	✓	✓	●	371.1880	-1.3	para-Chl...	20.8
Tox Sample: Brorphine...	Unknown	Methamphetamine	N/A	✓	✓	✓	✓	150.1271	-4.3	Metham...	95.3
Tox Sample: Brorphine...	Unknown	N-Propylamphetamine	N/A	✓	✓	✓	●	178.1582	-4.9	N-Propyl...	20.9
Tox Sample: Brorphine...	Unknown	Memantine	N/A	✓	✓	✓	▲	180.1742	-2.6	Memanti...	67.2
Tox Sample: Brorphine...	Unknown	Caffeine	N/A	✓	✓	✓	✓	195.0874	-1.4	Caffeine	99.4
Tox Sample: Brorphine...	Unknown	Morphine	N/A	✓	✓	✓	▲	286.1433	-1.7	Morphine	31.5
Tox Sample: Brorphine...	Unknown	Fentanyl	N/A	✓	✓	✓	✓	337.2271	-0.9	Fentanyl	100.0
Tox Sample: Brorphine...	Unknown	Clonazolam	N/A	✓	✓	✓	✓	354.0747	-1.5	Clonazol...	94.3
Tox Sample: Brorphine...	Unknown	ortho-Fluorofentanyl	N/A	✓	✓	✓	✓	355.2174	-1.7	ortho-Flu...	92.5
Tox Sample: Brorphine...	Unknown	meta-Fluorofentanyl	N/A	✓	✓	✓	✓	355.2174	-1.7	meta-Flu...	89.3
Tox Sample: Brorphine...	Unknown	Haloperidol	N/A	✓	✓	✓	▲	376.1466	-2.0	Haloperi...	49.4
Tox Sample: Brorphine...	Unknown	Brorphine	6.732e-1	✓	✓	✓	✓	400.1009	-2.6	Brorphine	88.0
Tox Sample: Brorphine...	Unknown	Verapamil	N/A	✓	✓	✓	✓	455.2896	-1.9	Verapamil	93.4

Sample Name	Sample Type	Component Name	Calculated Concentra ...	Mass Error ...	RT Confi...	Isotope Confi...	Library Confi...	Found At Mass	Mass Error (...	Librar ...	Library Score
Tox Sample: Brorphine...	Unknown	para-chlorofentanyl	< 0	✓	✓	✓	✓	371.1879	-1.6	para-Chl...	86.1
Tox Sample: Brorphine...	Unknown	Methamphetamine	N/A	✓	✓	✓	✓	150.1275	-1.2	Metham...	97.1
Tox Sample: Brorphine...	Unknown	N-Propylamphetamine	N/A	✓	✓	✓	✓	178.1586	-2.3	N-Propyl...	99.4
Tox Sample: Brorphine...	Unknown	Memantine	N/A	✓	✓	✓	✓	180.1746	-0.4	Memanti...	97.8
Tox Sample: Brorphine...	Unknown	Caffeine	N/A	✓	✓	✓	✓	195.0876	-0.1	Caffeine	99.4
Tox Sample: Brorphine...	Unknown	Morphine	N/A	✓	✓	✓	✓	286.1435	-1.0	Morphine	78.6
Tox Sample: Brorphine...	Unknown	Fentanyl	N/A	✓	✓	✓	✓	337.2274	-0.1	Fentanyl	100.0
Tox Sample: Brorphine...	Unknown	Clonazolam	N/A	✓	✓	✓	✓	354.0747	-1.5	Clonazol...	86.1
Tox Sample: Brorphine...	Unknown	ortho-Fluorofentanyl	N/A	✓	✓	✓	✓	355.2177	-0.9	ortho-Flu...	97.4
Tox Sample: Brorphine...	Unknown	meta-Fluorofentanyl	N/A	✓	✓	✓	✓	355.2177	-0.9	meta-Flu...	94.5
Tox Sample: Brorphine...	Unknown	Haloperidol	N/A	✓	✓	✓	✓	376.1467	-1.8	Haloperi...	93.0
Tox Sample: Brorphine...	Unknown	Brorphine	7.611e-1	✓	✓	✓	✓	400.1010	-2.2	Brorphine	99.4
Tox Sample: Brorphine...	Unknown	Verapamil	N/A	✓	✓	✓	✓	455.2896	-1.8	Verapamil	98.2

Figure 6. Results comparison between Zeno trap on and off for authentic postmortem case study #2. (Top) Results table and representative TOF MS/MS spectra with (top) and without (bottom) the Zeno trap enabled. The use of the Zeno trap resulted in a 10x improvement, on average, in sensitivity, which resulted in greater confidence in analyte identification confirmation through MS/MS spectral library matching.

Case study 2

The use of the Zeno trap for this qualitative workflow should provide substantial improvements in the observed TOF MS/MS spectral quality which should ultimately result in greater confidence in spectral library matching confirmation. Figure 6 (left) shows the results table from the analysis of discarded authentic postmortem case sample # 2 without (top) and with (bottom) activation of the Zeno trap. The analysis of this sample with the Zeno trap on resulted in greater library confidence for the majority of the positively identified compounds, as evidenced by comparing the green icons (bottom table) with the Zeno trap on to the red and yellow icons (top table) with the Zeno trap off.

For example, the library score for the identification of I-chlorofentanyl and N-propylamphetamine (ISTD) increased from 20.8% to 86.1% and from 20.9% to 99.4%, respectively, when the Zeno trap was activated. This drastic improvement in library score is the consequence of the MS/MS sensitivity enhancements afforded by the Zeno trap, which resulted in improved TOF MS/MS spectral quality. The sensitivity gains are shown in the TOF MS/MS spectra comparison for *para*-chlorofentanyl and N-propylamphetamine in Figure 6 (right). Overall, the average library score for the positively identified analytes in this sample increased from 74.5% to 94.4% when the Zeno trap was activated. It also resulted in a 10x improvement, on average, in sensitivity, which resulted in greater confidence in analyte identification confirmation through MS/MS spectral library matching.

Case study 3

Figure 7 shows the results tables and representative TOF MS/MS spectra comparison from the analysis of discarded authentic postmortem case sample #3. Similar observations can be drawn from the observations made for the analysis of the first case sample. First, the use of the Zeno trap resulted in the confident detection of all ten compounds with high confidence as evidenced by the high library scores ranging from 77.3 to 99.3%. Without the Zeno trap activated, analysis of this case sample resulted in two poorly matched analytes (*para*-chlorofentanyl and morphine returned a yellow and red library match icon, respectively) and three unmatched analytes (N-propylamphetamine, memantine and 5-aminoisonitazene returned a red library match icon) because of the poor quality of the triggered TOF MS/MS spectra. This is evidenced by comparing the TOF MS/MS spectra for two of these analytes, memantine and 5-aminoisonitazene (right). Without the Zeno trap activated, the generated TOF MS/MS spectra did not contain unique fragment ions to yield a library match. Overall, a 9x improvement, on average, in sensitivity was observed across the TOF MS/MS spectra positively identified in the three authentic postmortem case samples analyzed when the Zeno trap was activated (Figure 1). In addition, the use of the Zeno trap enabled acquisition of a much richer MS/MS spectrum that was used for confident compound identification, which resulted in an average library score increase from 56.3% to 88.6%.

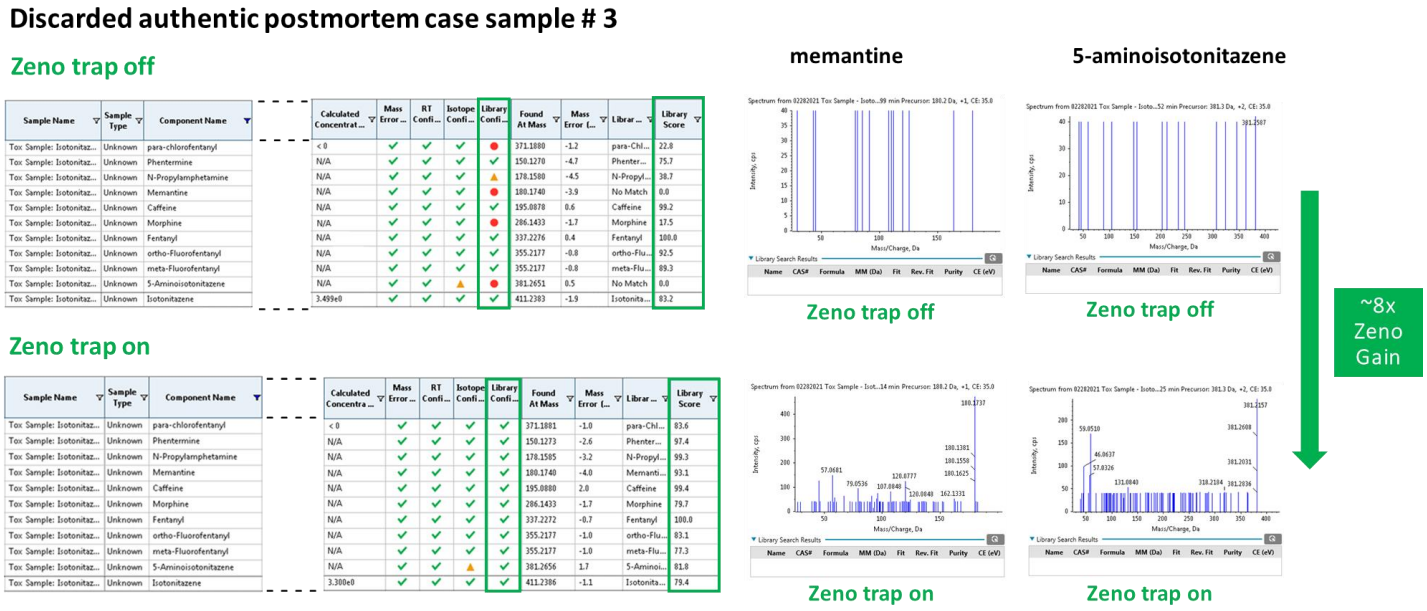


Figure 7. Results comparison between Zeno trap on and off for authentic postmortem case study #3. (Top) Results table and representative TOF MS/MS spectra with (top) and without (bottom) the Zeno trap enabled. The use of the Zeno trap enabled acquisition of a much richer MS/MS spectra that contained unique fragment ions that were used for confident compound identification.

A few observations can be drawn from the results highlighted in Figure 7. First, the added MS/MS sensitivity afforded by use of the Zeno trap enabled the accurate identification of 5-aminoisonitazene, one of metabolites of the potent NSO isotonitazene, with a library score of 81.8%. Second, the detection of fentanyl and other fentanyl analogs (*para*-chlorofentanyl and *para*-/*meta*-fluorofentanyl) suggest that the drug ingested by the subject might have originated from the illicit market. Although the presence of fentanyl might have been a contributing factor to the accidental overdose, the presence of the potent NSO isotonitazene and its metabolite could support the case of combined opioid drug toxicity leading to death.

Conclusions

A comprehensive and highly sensitive method for the screening and identification of potent NSO in human whole blood is described. The significant gains in MS/MS sensitivity on the ZenoTOF 7600 system yielded an improvement in confident identifications of low-level analytes through spectral library matching. The observed sensitivity gains afforded by the use of the Zeno trap resulted in a 9x improvement, on average, in TOF MS/MS sensitivity across the drugs positively identified in the authentic case samples analyzed. This improvement enabled confident identification of key drugs and metabolites at trace levels that were not previously achievable.

The MS/MS sensitivity levels afforded by ZenoTOF 7600 system provide a means to monitor low levels of ultra-potent NSO in poly-drug intake scenarios. This advancement could support the case of combined opioid drug toxicity leading to death, which offers a valuable insight into the causation of accidental overdoses.

References

1. Qualitative flexibility combined with quantitative power. SCIEX technical note, RUO-MKT-02-13053-A.
2. vMethod Application – Single-Injection Screening of 664 Forensic Toxicology Compounds on a SCIEX X500R QTOF System.

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