Establishing a qualitative and quantitative method for 803 poisons in blood and urine using the ZenoTOF 7600 system

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ABSTRACT

In this poster, the ZenoTOF 7600 system was used to establish a qualitative and quantitative method for the determination of 803 poisons in blood and urine. Compounds screened included drugs, alkaloids, pesticides, sedatives and rodenticides. The use of the Zeno trap, increased MS/MS intensity of the compound 4-20x, depending on the fragment ion m/z. This sensitivity increase improved the quality of the acquired MS/MS spectra, including the spectra acquired at the lower limit of quantification. This sensitivity enhancement improved sensitivity limits and the qualitative and quantitative accuracies for low-level detection of the targeted compounds. The use of electron-activated dissociation (EAD) for MS/MS provided different secondary fragment information, orthogonal to that obtained using collision-induced dissociation (CID) for the analytes and permitted accurate fragment structure conformation of compounds.

INTRODUCTION

Timely and comprehensive poison and drug screening in postmortem investigations challenges forensic laboratories because of the complexity of the samples. In addition, many compounds must be tested and new substances continuously enter the recreational drug market. Common drugs, pesticides, rat poisons, therapeutics, hypnotic sedatives and more comprise a broad set of compounds that should be screened to determine the cause of death. High-resolution mass spectrometry (HRMS) offers forensic laboratories a powerful tool to detect poisons and drugs, as it can detect thousands of compounds per analysis and efficiently and confidently identify them using MS and MS/MS data with spectral library matching. In this poster, we present a qualitative and quantitative workflow for the determination of poisons and drugs in blood and urine samples.

MATERIALS AND METHODS

Poisons and drugs in blood and urine were extracted by protein precipitation. Samples were analyzed using the ZenoTOF 7600 system (Figure 1) coupled with an ExionLC system. Poisons and drugs were chromatographically separated using a Phenomenex Luna Omega Polar C18 (2.1 x 100 mm, 1.6 µm) column. The composition of mobile phases A and B were 2 mM ammonium formate with 0.1% formic acid in water or methanol, respectively. The total LC runtime was 25 minutes. Mass spectrometric detection was conducted on a ZenoTOF 7600 system operated in both positive and negative electrospray ionization (ESI) modes. The MRM^{HR} workflow was used to investigate the advantages of the Zeno trap and EAD fragmentation on the quality of MS/MS spectra for the identification and quantification of compounds in matrix. Data processing was performed using SCIEX OS software, version 2.1 (Figure 2).

Figure 1. The ZenoTOF 7600 system.

Figure 2. SCIEX OS software, version 2.1.

Table 1. Mobile phase gradients used for the LC separation.

Time (min)	A (%)	B((%)
0.00	95	5
0.50	95	5
1.00	70	30
12.00	40	60
19.00	2	98
22.00	2	98
22.10	95	5
25.00	95	5

RESULTS

In this study, the ZenoTOF 7600 system was used to establish a qualitative and quantitative method for postmortem investigations to determine 803 poisons in blood and urine matrices. The target analytes investigated included 229 narcotic and psychotropic drugs, 69 hypnotic sedative drugs, 132 pesticides, 238 therapeutic drugs, 35 alkaloids and plant toxins, 13 rat poisons and 86 others (Figure 3).

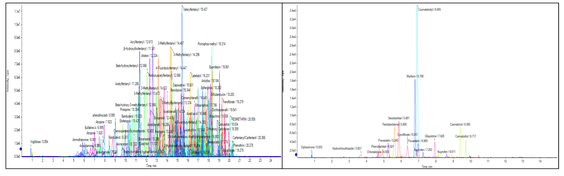

Figure 3. Extracted ion chromatogram (XIC) traces of 803 poisons acquired in positive (left) and negative (right) electrospray ionization (ESI) modes.

The Zeno trap is a key innovation on the ZenoTOF 7600 system that improves the MS/MS duty cycle in the orthogonal injection region of the QTOF, improving MS/MS sensitivity from 4- to 20-fold, depending on the m/z of the analytes. Here, the impact of the Zeno trap on compound identification was investigated. The MS/MS response intensity of the targeted compounds was increased when the Zeno trap was enabled. MS/MS intensity of the alkaloid compounds increased by ~10-20x on average when the Zeno trap was used (Figure 4). The quality of the MS/MS spectra obtained at the lower limit of quantification (LLOQ) was also significantly improved (Figure 5).

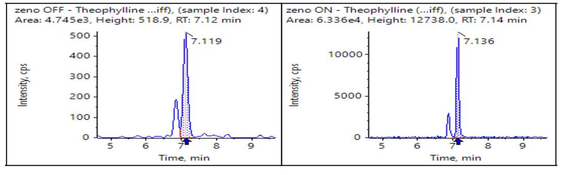

Figure 4. XIC traces showing the detection of theophylline with the Zeno trap off (left) and the Zeno trap on (right).

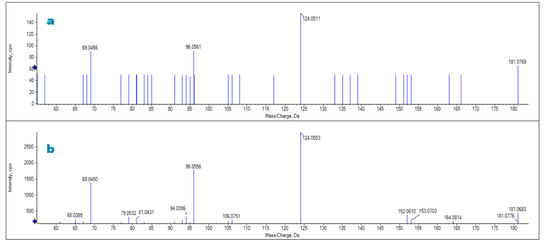

Figure 5. TOF-MS/MS spectra showing the detection of theophylline with the Zeno off (a) and the Zeno trap on (b).

EAD is an alternative fragmentation mode that produces MS/MS fragment information for compounds that differs from the structural information provided by CID. CID analysis of 4-fluorobutyrylfentanyl generated fragments at m/z 105.0699, 188.1436 and 248.1445. With EAD fragmentation, the fragments observed from CID were generated, in addition to fragments at m/z 164.0900, 207.1327, 277.1746 and 298.1880 (Figure 6). The combination of fragments generated with EAD more accurately characterized the compound.

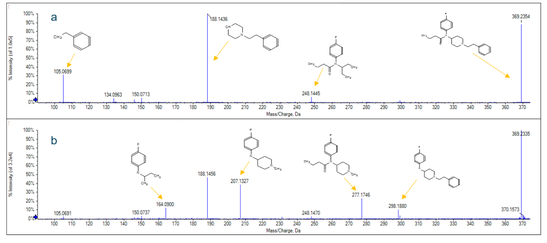

Figure 6. Detection of 4-fluorobutyrylfentanyl with CID (a) and EAD (b).

The recovery of the sample preparation was assessed by adding the compounds to blood and urine at 3 concentration levels. The results showed that the recovery of the analytical method ranged from 65% to 130%, with a relative standard deviation between 1.1% and 4.8%.

Overall, the presented method enabled the accurate qualitative and quantitative determination of 803 poisons and drugs in blood and urine.

CONCLUSIONS

A fast, robust and reliable method for the detection of 803 poisons in blood and urine matrices was developed. A generic extraction procedure was used to cover the large panel of analytes. High-resolution LC using a small particle size column was combined with high-sensitivity detection using the ZenoTOF 7600 system. When the Zeno trap was turned on, the response intensity of MS/MS was significantly increased, improving both the qualitative and quantitative accuracies of the assay. EAD produced complementary diagnostic fragment ions compared to CID, enabling more definitive characterization of compound structures.

The method was validated in blood and urine biological matrices. The recovery of the analytical method ranged from 65% to 130%, with a relative standard deviation between 1.1% and 4.8%. The features on the ZenoTOF 7600 system enabled accurate qualitative and quantitative determination of 803 poisons and drugs in blood and urine.

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