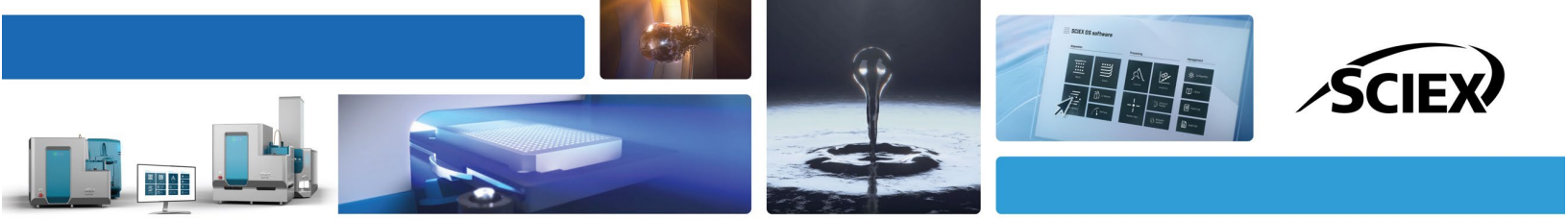

High-speed compound quality assessment using Acoustic Ejection Mass Spectrometry

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During drug discovery, high-throughput screening (HTS) is commonly used to identify bioactive compounds and campaigns use libraries containing thousands or millions of compounds. During storage, these compounds are typically dissolved in dimethyl sulfoxide (DMSO) and kept in 384- or 1536-well microtiter plates.[1] Confirming the identity of candidate compounds is critical to ensure they have been synthesized correctly and have not undergone degradation. Although LC-MS/UV and nuclear magnetic resonance (NMR) spectroscopy precisely identify compounds, long analytical runtimes and the need for expensive deuterated solvents make these techniques inefficient and expensive in high-throughput settings.[1,2]

This technical note demonstrates the rapid identification of 45 compounds using the Echo® MS+ system with ZenoTOF 7600 system using both polarities in less than 4 minutes (Figure 1).

Key benefits of the Echo® MS+ system with ZenoTOF 7600 system

- **Rapid analyte screening:** Screening of 45 compounds was completed within 4 minutes for positive and negative polarity
- **DMSO-based sample analysis:** Direct analysis of compounds dissolved in DMSO
- **Confident identification:** Identification of analytes using a combination of mass accuracy and isotope ratio pattern
- **384- and 1536-well format support:** High-throughput screening of samples plated in either well format is supported
- **Streamlined acquisition, data processing and review:** SCIEX OS software provides support for all steps of data analysis, including automatic report generation

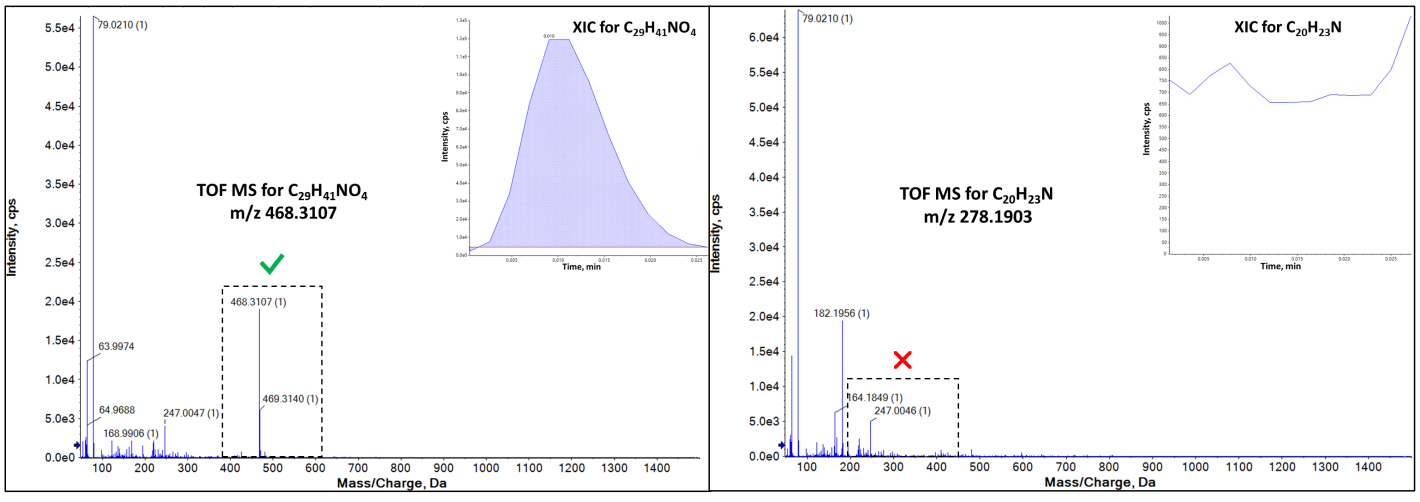


Figure 1. Confident identification of compounds. TOF MS data were acquired for 45 compounds dissolved in DMSO and plated on a 384-well microtiter plate. The TOF MS spectrum and extracted ion chromatogram (XIC) for $C_{29}H_{41}NO_4$ (left) and $C_{20}H_{23}N$ (right) are shown. Identification was supported by mass accuracy (< 5ppm) and isotope ratio difference (<20%).

Introduction

Drug discovery programs require the screening of sizable compound libraries to identify potential drug candidates. Screening large arrays of compounds in pharmaceutical, biotechnology and academic centers is typically accomplished using LC-MS/UV.[1] While fast 2.5-minute LC-MS/UV analysis methods have been developed, testing 2000 compounds still requires approximately 1 week.[2] Although NMR screening methods require small amounts of target compounds, expensive deuterated solvents are needed to solubilize related samples.[1]

Acoustic Ejection Mass Spectrometry (AEMS) utilizes the contactless sampling of nanoliter-size droplets, transfer to the Open Port Interface and analysis using the ZenoTOF 7600 system to provide rapid results with high mass accuracy. With a sampling speed of up to 1 sample per second, this platform screens samples within 10 minutes for a 384-well plate and in less than 30 minutes for a 1536-well plate.[3] These rapid screening rates enable fast decisions based on mass accuracy and isotope ratio difference in a high-throughput setting.

Users can select from 3 fluid classes for ejections using the Echo® MS+ system with ZenoTOF 7600 system. Aqueous (AQ), surfactant (SP) or DMSO fluids can be selected for ejection based on the nature of the sample matrix. Since most compounds are dissolved in DMSO and formatted in a 384- or 1536-well microtiter plate,[4] the end user can obtain compound identity by directly analyzing nanoliter-size droplets from these plates using this system.

Methods

Samples and reagents: The neat standards for 45 analytes in this study were purchased from Sigma Aldrich at a 1 mg/mL concentration in methanol. DMSO ACS reagent, ≥ 99.9%, was purchased from Sigma Aldrich.

Sample preparation: First, 990 µL of DMSO was added to 45 wells of a 96-well plate. To each well, 10 µL of an analyte was added to reach a final 10 µg/mL concentration. A 50 µL aliquot was transferred from each of these wells into an Echo® MS qualified 384-well plate. The plate was centrifuged at 4000 rpm for 10 minutes and shaken for 5 minutes before analysis.

Acoustic ejection: The carrier solvent was 0.1% formic acid in methanol. The flow rate was 350 µL/min with an ejection volume of 2.5 nL. A standard peak mode of 1 sample per second with a 1500 ms delay time between ejections was chosen for this method. The DMSO fluid class was selected for sample ejection.

Mass spectrometry: Tables 1 and 2 list the optimized source and gas parameters used for analysis in positive and negative polarity, respectively. The analytes in this panel cover positive and negative compound classes, therefore a TOF MS experiment was conducted with positive and negative ionization modes. Automated mass calibration of the mass spectrometer was performed to ensure that mass accuracy was maintained throughout batch acquisition.

Table 1. Source and gas parameters for positive polarity.

Parameter	Value
Polarity	*Positive*
Ion source gas 1	*90 psi*
Ion source gas 2	*60 psi*
Curtain gas	*35 psi*
Source temperature	*400°C*
Ion spray voltage	*5500 V*
CAD gas	*7*
MS method	*TOF MS*

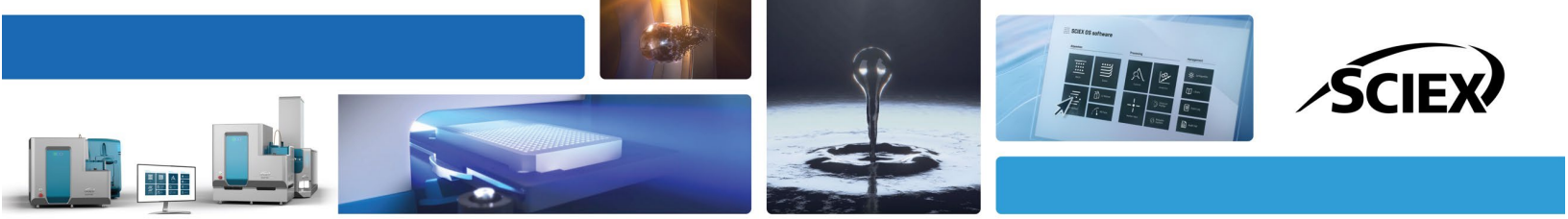
Table 2. Source and gas parameters for negative polarity.

Parameter	Value
Polarity	*Negative*
Ion source gas 1	*90 psi*
Ion source gas 2	*60 psi*
Curtain gas	*35 psi*
Source temperature	*400°C*
Ion spray voltage	*-4500 V*
CAD gas	*7*
MS method	*TOF MS*

Data processing: Data collection and analysis were performed using SCIEX OS software. Data review and processing were performed in the Explorer and Analytics modules of SCIEX OS software, respectively.

Rapid screening of compounds in a panel

To simulate a compound library setting, 45 compounds were selected that could be analyzed in positive or negative ionization modes. Deuterated internal standards for 2 analytes and a negative control were also included in this panel. A TOF MS acquisition experiment was performed to identify analytes in this study. The total ion chromatogram



(TIC) was analyzed for all compounds, including the barcodes present at the start and end of the run. SCIEX OS software uses the characteristic barcode pattern to align the timing data with ejections from the corresponding well positions in the sample plate. The extracted ion chromatogram (XIC) of each compound was reviewed with SCIEX OS software. The total acquisition was completed within 2 minutes for each polarity (Figure 2).

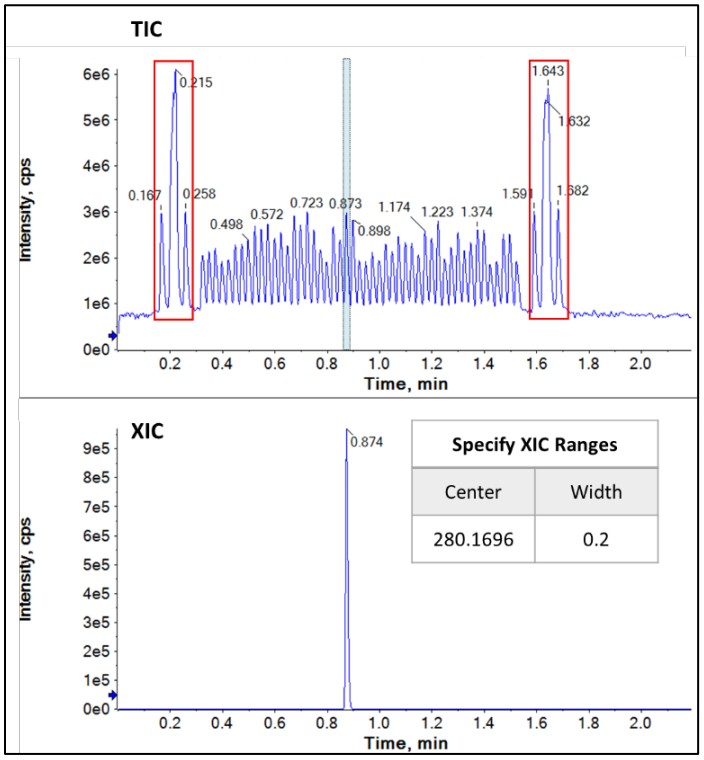


Figure 2. Total ion chromatogram (TIC) of the TOF MS data acquired in positive polarity (top). The barcodes present at the start and end of a run are highlighted in red. The XIC of an analyte at m/z 280.1696 was detected at 0.874 minutes (bottom).

Compound identification using SCIEX OS software

The post-acquisition processing method was built in the Analytics module of SCIEX OS software. The summation integration algorithm was used for its speed and consistent integration of non-chromatographic peaks. The integration parameters, precursor mass, chemical formula, adduct and charge were provided to build a processing method for the analyte at m/z 280.17 detected in the positive ionization mode. A similar processing method was built for the compound at m/z 343.19, which was evaluated in the negative ionization mode. Three confidence criteria were applied to identify the compounds: 1) the peak must be detected based on signal-to-noise (S/N) ratio, 2) the mass error must be <5 ppm and 3) the isotope ratio difference must be <20%. Peak area integration and visual evaluation of the TOF MS spectrum were conducted in the Analytics module of SCIEX OS software (Figure 3).

Since each well contained a different analyte, the TOF MS data were processed using the reprocessing tool in the Explorer module of SCIEX OS software. An editable text file, known as a target list, was created that contained the well position, chemical formula, adduct and charge of each compound in that plate. Using the target list, processing method and data file, the reprocessing tool generated a results text file containing the mass error (ppm), isotope ratio difference and confidence status. The reprocessing was performed for data acquired in both polarities. Once the initial processing method has been set, the automatically triggered processing capabilities of SCIEX OS software can be applied to process subsequent batches automatically. The mass accuracy and isotope ratio for 45 compounds were within the set confidence criteria (mass accuracy <5 ppm and isotope ratio difference <20%), confirming the identities of these compounds (Table 3). Degradation of compounds 37 and 43 caused them to fail the confidence criteria.

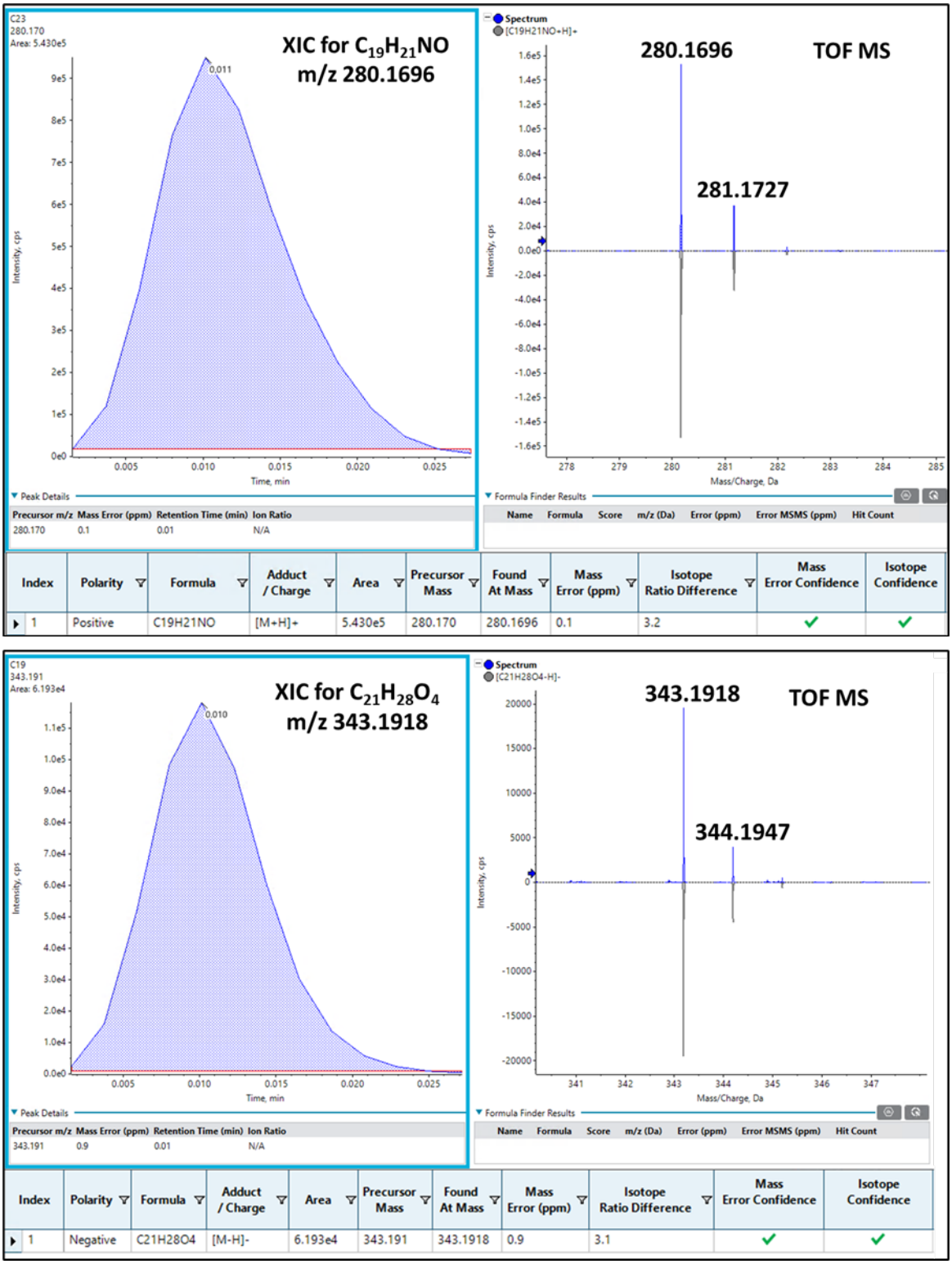


Figure 3. Representative XICs and TOF MS spectra for compounds detected using positive and negative ionization modes. The XIC and TOF MS spectrum are shown for the compound $C_{19}H_{21}NO$, which was detected using positive ionization mode (top). The XIC and TOF MS spectrum are shown for the compound $C_{21}H_{28}O_4$, which was analyzed using negative ionization mode (bottom). In each case, the Analytics module of SCIEX OS software generated a results table showing polarity, formula, peak area, adduct/charge, precursor and found mass. The mass error and isotope confidence of the compound were also determined, as indicated by the green checks.

Table 3. Compound information for the 45 analytes analyzed on the Echo® MS+ system with ZenoTOF 7600 system. Chemical formula, adduct/charge, precursor mass, mass error, mass error confidence, isotope ratio difference, isotope confidence and peak area are included. *Compounds 19, 20 and 44 were detected in the negative ionization mode. The peak area threshold was set for positive identification (>2e3). The analytes in this study were identified using the confidence criteria established for mass accuracy and isotope pattern match.

Compound number	Formula	Adduct / charge	Precursor, m/z (Error, ppm)	Mass error confidence	Isotope ratio difference	Isotope confidence	Peak area
1	$C_{29}H_{41}NO_4$	[M+H]+	468.3107 (0)	✓	1.5	✓	200790
2	$C_{18}H_{21}NO_3$	[M+H]+	300.1594 (-1)	✓	1.1	✓	163811
3	$C_{17}H_{19}NO_3$	[M+H]+	286.1438 (-0.5)	✓	1.5	✓	139983
4	$C_9H_{13}N$	[M+H]+	136.1121 (-2.6)	✓	0.5	✓	7614
5	$C_{10}H_{15}N$	[M+H]+	150.1277 (-2)	✓	2.7	✓	37360
6	$C_{11}H_{15}NO_2$	[M+H]+	194.1176 (-1.1)	✓	2.5	✓	97338
7	$C_{10}H_{13}NO_2$	[M+H]+	180.1019 (-2.1)	✓	3.0	✓	22691
8	$C_{16}H_{19}NO_4$	[M+H]+	290.1387 (0.4)	✓	2.1	✓	303750
9	$C_{10}H_{17}NO_3$	[M+H]+	200.1281 (-0.5)	✓	0.3	✓	516986
10	$C_{16}H_{13}ClN_2O$	[M+H]+	285.0789 (-0.3)	✓	1.6	✓	364982
11	$C_{15}H_{11}ClN_2O$	[M+H]+	271.0633 (-0.2)	✓	3.4	✓	405342
12	$C_{15}H_{11}N_2O_2Cl$	[M+H]+	287.0582 (-0.3)	✓	2.0	✓	176898
13	$C_{16}H_{13}ClN_2O_2$	[M+H]+	301.0738 (0.2)	✓	0.5	✓	143710
14	$C_{17}H_{13}ClN_4O$	[M+H]+	325.0851 (-0.4)	✓	0.8	✓	190754
15	$C_{15}H_{12}ClN_3O$	[M+H]+	286.0742 (0.4)	✓	3.6	✓	496764
16	$C_{16}H_{14}N_3OF$	[M+H]+	284.1194 (0.5)	✓	0.7	✓	592943
17	$C_{15}H_{13}N_3O$	[M+H]+	252.1131 (-0.9)	✓	3.0	✓	714862
18	$C_9H_{17}NO_2$	[M+H]+	172.1332 (-0.6)	✓	0.4	✓	202839
*19	$C_{21}H_{28}O_4$	[M-H]-	343.1918 (0.9)	✓	3.1	✓	61930
*20	$C_{11}H_{18}N_2O_3$	[M-H]-	225.1245 (0.5)	✓	2.1	✓	7215
21	$C_{25}H_{35}NO_4$	[M+H]+	414.2639 (0.4)	✓	3.3	✓	365930
22	$C_{17}H_{13}ClN_4$	[M+H]+	309.0902 (-0.1)	✓	4.3	✓	203931
23	$C_{19}H_{21}NO$	[M+H]+	280.1696 (0.1)	✓	3.2	✓	543014
24	$C_{15}H_{21}NO_2$	[M+H]+	248.1645 (-0.1)	✓	0.7	✓	585018
25	$C_{62}H_{111}N_{11}O_{12}$	[M+H]+	1202.8486 (0.8)	✓	3.5	✓	4203
26	$C_{44}H_{69}NO_{12}$	[M+H]+	804.4893 (0.3)	✓	2.0	✓	2797
27	$C_{18}H_{21}NO_4$	[M+H]+	316.1543 (-0.6)	✓	1.1	✓	143334
28	$C_{17}H_{14}ClFN_2O_2$	[M+H]+	333.0801 (-0.7)	✓	0.2	✓	130825
29	$C_{17}H_{19}N_3$	[M+H]+	266.1652 (-0.7)	✓	0.6	✓	291910
30	$C_{15}H_{12}N_2O_2$	[M+H]+	253.0972 (-1.1)	✓	3.5	✓	68087
31	$C_{24}H_{29}NO_9$	[M+H]+	476.1915 (-0.1)	✓	1.5	✓	173611
32	$C_{16}H_{25}NO_2$	[M+H]+	264.1958 (0)	✓	2.3	✓	209687
33	$C_{16}H_{14}ClN_3O$	[M+H]+	300.0898 (0.2)	✓	2.6	✓	193518
34	$C_{16}H_{17}NO_4$	[M+H]+	288.1230 (0)	✓	1.4	✓	229099

35	*$C_{18}H_{19}ClN_4$*	*[M+H]+*	*327.1371 (-0.4)*	✓	*1.8*	✓	*316900*
36	*$C_{16}H_{19}NO_2$*	*[M+H]+*	*258.1489 (-1.6)*	✓	*1.5*	✓	*5609*
37	*$C_{20}H_{23}N$*	*[M+H]+*	*278.1903 (-148.9)*	●	*8.1*	✓	*9*
38	*$C_{20}H_{21}N$*	*[M+H]+*	*276.1747 (-0.7)*	✓	*1.4*	✓	*502308*
39	*$C_8H_{17}NO_2$*	*[M+H]+*	*160.1332 (-1.5)*	✓	*1.5*	✓	*76284*
40	*$C_{15}H_{12}N_2O_2$*	*[M+H]+*	*253.0972 (-0.8)*	✓	*2.5*	✓	*86836*
41	*$C_{20}H_{23}NO_4$*	*[M+H]+*	*342.1700 (0.2)*	✓	*1.5*	✓	*441770*
42	*$C_{14}H_{19}NO_2$*	*[M+H]+*	*234.1489 (0)*	✓	*1.8*	✓	*526026*
43	*$C_9H_7D_{10}NO_2$*	*[M+H]+*	*182.2110 (-83.6)*	●	*N/A*	●	*1517*
**44*	*$C_{21}H_{25}D_3O_4$*	*[M-H]-*	*346.2100 (0.5)*	✓	*3.9*	✓	*64240*
45	*C_2H_6SO*	*[M+H]+*	*79.0212 (-2.7)*	✓	*1.7*	✓	*182204*

Conclusions

- A low sampling volume of 2.5 nL enabled the conservation of library samples for future testing
- Confident determination of 43 out of 45 compounds based on mass accuracy and isotope ratio guided informed decisions in a high-throughput setting
- Analysis of 45 compounds dissolved in DMSO and plated in a 384-well microtiter plate was achieved in <2 minutes for a single polarity, improving valuable throughput and compound savings

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