Accurate Mass Report Generation with Spectral Accuracy Isotope Scoring within a high throughput laboratory

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Overview

- Processing of high-resolution accurate-mass data at the EPSRC National Mass Spectrometry Facility (NMSF) has been a manual process due to the unique combination of the Advion TriVersa NanoMate and the Thermo Scientific LTQ Orbitrap XL
- Data was not processed satisfactory through automation by the native Xcalibur software.
- The NMSF sought a solution that would allow the generation of user reports efficiently, through the use of batch processing, that would give the user additional confidence in the mass spectrometry results by utilising spectral accuracy.
- Data submitted to AutoConfirm for processing showed a success rate of 88% initially. Samples that failed were found to be ionic in nature; details submitted by the user as a neutral formula were not reflected in the results that were obtained.
- After the target list was modified to account for non-neutral samples, the software correctly identified 98%. The software was modified to account for additional adduct ions, specifically seeking the adducts including H+, NH4+, Na+, K+.
- The reports generated display the full mass spectrum, an expansion of the m/z region of interest with a superimposed theoretical isotope pattern match.
- The isotope pattern is scored through spectral accuracy using a published algorithm.
- The lowest spectral accuracy is at 47.5% with a mean spectral accuracy of 96.1% from the submitted samples which contained a variety of elements; C, H, N, O, Sn, P, Cu, F, Ir, Br of neutral molecular weights from 265 Da to 1446 Da

Introduction

- Accurate mass measurement is an invaluable tool for the identification of chemicals.
- It is well known that accurate mass measurement will provide a unique compound I.D. for samples with a molecular weight less than 300 Da with only C, H, N and O as atoms. Above this, the number of matches to a given molecular weight increases.
- To aid structural identity, mass spectrometrists can utilise additional information obtained within the sample spectrum, namely isotope patterns.
- Using an established algorithm, the authors demonstrate how effective the combination of spectral accuracy[1] (isotope scoring) and accurate mass measurements are for the confirmation of known target molecules in an automated fashion with a Thermo Scientific Orbitrap mass spectrometer.

Methods

- Samples were submitted to the EPSRC NMSF, Swansea University, Wales, by external users via a web based sample submission.
- Samples were prepared as per sample preparation guidelines[1].
- The samples were deposited into a 96-well plate for analysis upon an Advion TriVersa NanoMate (Advion, Itheca, NY) and a LTQ Orbitrap XL (Thermo Scientific, San Jose, CA).
- LTQ-Orbitrap calibration mixes (CalMix) were prepared as per the manufacturer's instructions[3] for positive ion mode.
- Data was acquired at a resolution R=30,000 via infusion with the TriVersa NanoMate.
- A comma delimited file was exported from the Xcalibur sequence list containing Filename and Expected Formula (provided upon submission of the sample to the NMSF via a web base submission).
- The bespoke software, AutoConfirm, was created to automatically process the data and generate reports

Results

- Table 1 shows the summary output from AutoConfirm.
- The target spectral accuracy was set to 90%; only two samples fell outside of this
- The filenames are hyperlinks which directs the user to a portable document format (pdf) file (Figure 1)
- Samples with complex isotope patterns scored good Spectral Accuracy, e.g. CERNO025 – CERNO028

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Id	FileName	Molecular Formula	Mod	Detected m/z	SA(%)	m/z err(ppm)	m/z err(mDa)
1	CERNO001	C16H27N3O3S2	+H	374.1571	96.11	1.2	0.4
2	CERNO002	C16H21N3O2S2	+H	352.1152	95.06	1.2	0.4
3	CERNO003	C32H40N6O4S4	+Na	723.1880	90.34	-0.8	-0.6
4	CERNO004	C21H25NO2	+H	324.1963	98.78	1.7	0.5
5	CERNO005	C23H20O2	+H	329.1541	98.75	1.4	0.5
6	CERNO006	C21H31N3O3S2	+H	438.1877	96.72	-0.5	-0.2
7	CERNO007	C20H41NO3Si	+H	372.2930	97.66	0.5	0.2
8	CERNO008	C14H25N3O3S	+H	316.1692	97.79	0.9	0.3
9	CERNO009	C18H25N3O3S	+H	364.1694	97.00	1.3	0.5
10	CERNO010	C23H25NO3S	+H	396.1635	98.85	1.8	0.7
11	CERNO011	C15H27NO4Si	+H	314.1789	98.17	2.2	0.7
12	CERNO012	C22H30F3NO4Si	+H	458.1974	95.52	1.0	0.5
13	CERNO013	C13H15BrN2O	+H	295.0444	96.52	1.2	0.3
14	CERNO014	C23H27NO3	+H	366.2067	98.83	0.9	0.3
15	CERNO015	C18H32O5Si	+H	357.2098	97.82	1.7	0.6
16	CERNO016	C17H32O5Si	+H	345.2096	97.62	1.3	0.4
17	CERNO017	C27H46O6Si	+H	495.3132	91.09	-0.8	-0.4
18	CERNO018	C67H119NO9Si2Sn	+H	1250.7514	89.97	-2.4	-3.0
19	CERNO019	C64H100NO12PSi2	+H	1162.6591	94.97	-0.3	-0.4
20	CERNO020	C56H103NO8Si3	+H	1002.7074	96.08	0.9	0.9
21	CERNO021	C29H33N2O2	-e	441.2544	98.71	1.6	0.7
22	CERNO022	C29H35N2	-e	411.2800	98.26	1.3	0.5
23	CERNO023	C26H27N3O2	+H	414.2179	98.98	0.6	0.3
24	CERNO024	C25H27N3O	+H	386.2231	98.20	0.9	0.4
25	CERNO025	C27H29N5Cl2Cu	-Cl	521.1402	95.49	0.0	0.0
26	CERNO026	C54H58N10Cu(ClO4)2	-ClO4	1008.3628	96.96	0.6	0.6
27	CERNO027	C27H29N5Cu(N3)2	-(N3)2	486.1713	96.40	0.0	0.0
28	CERNO028	C27H29N5Cu(NCS)2	-NCS	544.1459	96.83	-1.0	-0.6
29	CERNO029	C17H14IOS	-e	392.9801	99.24	-1.0	-0.4
30	CERNO030	C20H18IO2	-e	417.0340	99.36	-1.4	-0.6
31	CERNO031	C19H16IO	-e	387.0236	99.28	-1.1	-0.4
32	CERNO032	C23H22INO4	+H	504.0661	99.40	-1.1	-0.6
33	CERNO033	C62H56Cl2F4Ir2N6O2	-Cl	1409.3290	91.44	-0.4	-0.5
34	CERNO034	C42H44O22	+H	901.2398	97.28	0.2	0.1
35	CERNO035	C12H20O7S	+H	309.1003	98.39	0.1	0.0
36	CERNO036	C17H19NO2	+H	270.1490	98.90	0.5	0.1
37	CERNO037	C14H18ClF6NO	-Cl	330.1294	99.27	1.9	0.6
38	CERNO039	C21H20F6N2OS	+H	463.1271	98.00	-0.4	-0.2
39	CERNO040	C12H18ClF2NO	-Cl	230.1351	99.39	0.0	0.0
40	CERNO041	C19H20F2N2OS	+H	363.1344	98.52	1.9	0.7
41	CERNO042	C21H25NO4	+Na	378.1673	47.50	-0.7	-0.3
42	CERNO043	C22H24O4	+H	353.1752	98.75	1.2	0.4
43	CERNO044	C26H21F3O4	+H	455.1463	99.30	-0.4	-0.2
44	CERNO045	C47H55NO8	+H	762.4002	99.15	0.1	0.1
45	CERNO046	C48H57NO9	+H	792.4105	98.74	-0.1	-0.1
46	CERNO047	C49H59NO10	+H	822.4205	98.07	-0.9	-0.7
47	CERNO048	C48H61NO9	+H	796.4438	94.00	2.4	1.9
48	CERNO049	C70H94N2O12	+H	1155.6871	98.10	-0.7	-0.9
49	CERNO050	C46H57NO8	+H	752.4167	96.53	1.3	1.0
50	CERNO051	C49H63NO8	+Na	816.4443	91.79	-0.4	-0.3

Table 1: Summary table from AutoConfirm showing target formula, expected modification (Mod), detected m/z, Spectral Accuracy (SA), and m/z error.

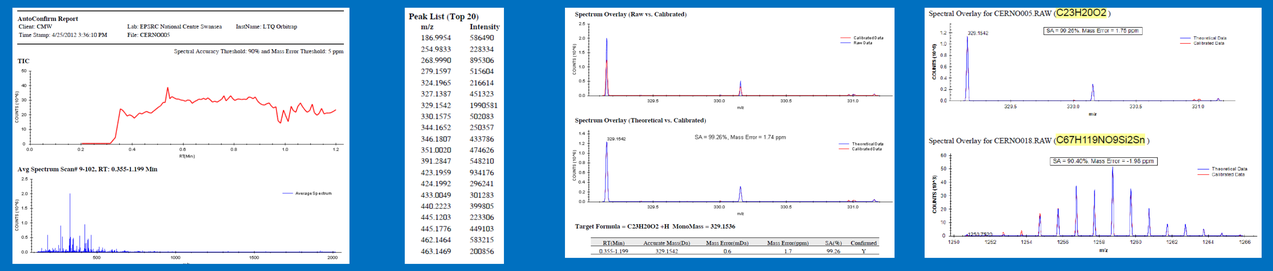

Figure 1: Output from AutoConfirm for sample CENRO005. From left to right: Total Ion Current (top) and Averaged Spectrum, RT 0.36 to 1.2 minutes (bottom); Peak list of top 20 ions observed in Averaged Spectrum; Spectrum Overlay for both Raw vs. Calibrated (top) and Theoretical vs. Calibrated (bottom); Spectral Overlay for simple isotope compound (CERNO005, top) and complex isotope compound (CERNO018, bottom).

Conclusions

- For Orbitrap data the software has proved to be very fast for automated analysis and report generation (approx. 1 minute for 50 samples)
- The software runs at the end of an acquisition, batch processing all the files from an exported Xcalibur sequence list
- The operator has a summary page which highlights samples that have falling outside of the specified targets (Table 1)
- The end user has an automatically generated pdf file containing the results (Figure 1)
- The combination of both accurate mass and Spectral Accuracy provides increased confidence for compound assignment

References

1. "The Concept of Spectral Accuracy for MS", Y. Wang and M. Gu, *Anal. Chem.* **82** (2010) pp7055-7062
2. EPSRC NMSF SOP, http://nmsf.swan.ac.uk/
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