Guidelines for data submission into the Unified CCS Compendium

Single Field Data

The supplemental information packet includes a file entitled "SI_SingleField_DataFormat.xlsx". The two spreadsheets ("Single Field Reference Standards" and "Single Field Data Format") within the Excel file will need to be populated prior to submission of single field data to the unified CCS Compendium. *Caution: If the Excel file is opened in read-only mode, the spreadsheet will not be editable. Please click 'enable editing' to proceed.*

Step 1: Collect, at minimum, triplicate measurements of the reference standards for each day samples are acquired.

- Recommended strategy: Infuse reference standards simultaneously with the analyte(s) of interest (i.e., as an internal reference). This allows the reference standards to be measured under the same conditions that the analytes are exposed to.
- If measuring reference standards independently, it is advised to acquire CCS measurements of the reference standards before, during, and after each set of acquisitions to assess any systematic deviations in mass and mobility measurements. This will also allow profiles of pressure, temperature, and electric field to be constructed for each acquisition set, to assist in assessing measurement quality.

Step 2: Collect, at minimum, triplicate measurements of the experimental analytes of interest (if not acquired in step 1). Include $\geq$ 5 compounds from the quality assessment (QA) compounds list (Table S1g) to assess data.

- Experimental values for analytes chosen from the QA compounds list must meet the following criteria:
  - Average CCS percent error of $\leq$ 0.5%

$$percent\ error = \frac{(CCS_{experimental} - CCS_{QA})}{CCS_{QA}} \cdot 100$$

  - Maximum individual CCS percent error $\leq$ 1%

Step 3: Populate columns A-F in the spreadsheet entitled "Single Field Reference Standards" (see Fig. S1a) with experimental data generated from step 1 for each replicate.

- Use rows 5-14 for positive ion data and/or rows 15-24 for negative ion data.
- Single-field CCS and corresponding m/z values can be obtained using the "CCS Calibration (Single-Field)" method implemented in IM-MS Browser (Agilent Technologies). Alternately, the single-field drift time/CCS relationship can be calculated directly from drift time measurements of reference standards using equations described in previous work.[2]
- Columns G-M in the spreadsheet will be auto-populated. Important: The data in this spreadsheet is intended to be used ONLY for reference standards that were measured, NOT the analytes being submitted to the CCS Compendium.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
4	Experimental Replicate 1 m/z	Experimental Replicate 2 m/z	Experimental Replicate 3 m/z	Experimental Replicate 1 CCS	Experimental Replicate 2 CCS	Experimental Replicate 3 CCS	Reference Standards m/z	Reference Standards CCS	Experimental Average m/z	m/z error (ppm)	Experimental Average CCS	CCS Std Dev	CCS % RSD	% CCS Difference	Polarity
5	118.086	118.088	118.085	121.40	121.30	121.32	118.086	121.30	118.09	2.82	121.34	0.05	0.04%	0.03%	+
6	322.048	322.050	322.047	153.80	153.73	153.75	322.048	153.73	322.05	1.04	153.76	0.04	0.02%	0.02%	+
7	622.029	622.031	622.028	203.00	202.96	202.98	622.029	202.96	622.03	0.54	202.98	0.02	0.01%	0.01%	+
8	922.010	922.012	922.009	243.70	243.64	243.66	922.010	243.64	922.01	0.36	243.67	0.03	0.01%	0.01%	+
9	1221.991	1221.993	1221.990	282.30	282.20	282.22	1221.991	282.20	1221.99	0.27	282.24	0.05	0.02%	0.01%	+
10	1521.971	1521.973	1521.970	317.10	316.96	316.98	1521.971	316.96	1521.97	0.22	317.01	0.08	0.02%	0.02%	+
11							1821.952	351.25							+
12							2121.933	383.03							+
13							2421.914	412.96							+
14							2721.895	441.21							+
15							112.986	108.23							-
16							301.998	140.04							-
17							601.979	180.77							-
18							1033.969	255.34							-
19							1333.969	284.76							-
20							1633.950	319.03							-
21							1933.931	352.55							-
22							2233.911	380.74							-
23							2533.892	412.99							-
24							2833.873	432.62							-
25											Averages:	0.04	0.02%	0.02%	

Figure S1a. A screenshot of the "Single Field Reference Standards" spreadsheet

Step 4: Populate Columns A-K of the spreadsheet entitled "Single Field Data Format" (see Fig. S1b) with experimental data acquired in step 2.

- Columns L-P will be auto-populated.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Compound	Formula	CAS	Adduct	Charge	Experimental Replicate 1 m/z	Experimental Replicate 2 m/z	Experimental Replicate 3 m/z	Experimental Replicate 1 CCS	Experimental Replicate 2 CCS	Experimental Replicate 3 CCS	Average Experimental m/z	Average Experimental CCS	Std. Dev	%RSD	CCS.z
2	Example Lipid	C45H73NO8P	5634-86-6	[M-H]	-1	786.5070	786.5074	786.5109	277.30	277.33	277.27	μ m/z	μ CCS	σ	=σ/μCCS*100	277.30
3	(R)-Malate	C4H6O5	636-61-3	[M-H]	-1	133.0137	133.0151	133.0128	120.08	121.1	120.45	133.01	120.54	0.52	0.43	0.91
4	Your	Data	Here!													

Figure S1b. "Single Field Data Format" spreadsheet (Columns A-K)

Step 5: Classify each compound using the ClassyFire web application (found at http://classyfire.wishartlab.com/).[3]

- Populate Columns Q-V of the spreadsheet entitled "Stepped Field Data Format" (shown in Fig. S1c) with the classification information, source (e.g. research group), and DOI (if published).

	A	B	C	D	E	Q	R	S	T	U	V
1	Compound	Formula	CAS	Adduct	Charge	Kingdom	Super.Class	Class	Subclass	Source	DOI
2	Example Lipid	C45H73NO8P	5634-86-6	[M-H]	-1	Organic compounds	Lipids and lipid-like molecules	Glycerophospholipids	Glycerophosphoethanolamines	Research Group	If unpublished, please fill in as "Unpublished".
3	(R)-Malate	C4H6O5	636-61-3	[M-H]	-1	Organic compounds	Lipids and lipid-like molecule	Fatty acyls	Fatty acids and conjugates	McLean	Unpublished
4	Your	Data	Here!			Your	Classifications	Here!			

Figure S1c. "Single Field Data Format" spreadsheet (Columns Q-V)

Step 6: Calculate the average RSD for *all* experimental values (QA compounds as well as all analytes/compounds that are being submitted for inclusion into the unified CCS compendium).

- The CCS values submitted must meet the following criteria:
  - Average RSD $\leq$ 0.5% for the full experimental data set
  - Individual compound RSD $\leq$ 0.7%

Step 7: Double check to ensure that steps 1-6 were performed.

- Step 1: Triplicate measurements acquired for reference standards each day sample measurements were collected.
- Step 2: Triplicate measurements acquired for ***all*** experimental values, including at least five compounds from the QA compound list.
- Step 3 & 4: Enter all data into the formatted spreadsheets.
- Step 5: Classify all compounds in the provided columns of the spreadsheets.
- Step 6: Calculate average RSD and individual RSD.

Step 8: Submit spreadsheet for quality assessment.

Data must be submitted by emailing the completed spreadsheet ("SI_SingleField_DataFormat.xlsx") to ccscompendium@vanderbilt.edu.

- Please include the following information with each submission.
  - Institution
  - Research group
  - Instrument source type
  - Solvent/buffer system
  - List of reference compounds included in experimental data set