Guidelines for data submission into the Unified CCS Compendium

Stepped Field Data

The supplemental information packet includes a file entitled "SI_SteppedField_ScaleAndDataFormat.xlsx". The two spreadsheets ("Stepped Field Reference Standards and Scale" and "Stepped Field Data Format") within the Excel file will need to be populated prior to submission of stepped 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.

- Ideal strategy: Infuse reference standards simultaneously with the analyte(s) of interest (i.e., as 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, acquire reference standards CCS measurements before, during, and after each set of acquisition to assess drift in mass and mobility measurements.

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

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

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

  - Maximum individual CCS percent error ≤ 1%

Step 3: Populate columns A and B in the spreadsheet entitled "Stepped Field Reference Standards and Scale" (see Fig. S1d) with data generated from step 1 for each replicate.

- True effective lengths for data collected in Step 1 must be calculated using the "Stepped Field Reference Standards and Scale" spreadsheet. Further detail addressing the purpose of scaling and the scaling procedure are discussed in supplemental Fig. S2.
- Use rows 7-16 for positive ion mode and/or rows 17-26 for negative ion mode.
- CCS and m/z values can be obtained using the "CCS Calculator (Stepped-Field)" method in IM-MS Browser (Agilent Technologies). Alternately, stepped-field CCS values can be calculated from corrected drift times using the fundamental low-field ion mobility equation.[4,5] Drift time correction requires a linear regression analysis incorporating the raw drift time measured at each of the drift fields surveyed, as described previously.[6]
- Columns C-G in the spreadsheet will be auto-populated.
- Important: The data in this spreadsheet is 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	P
4	Experimental Effective Length (cm):			78.24												
5																
6	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 Standard m/z	Reference Standard CCS	Experimental Average m/z	mz error (ppm)	Experimental Average CCS	CCS Std Dev	CCS % RSD	Scale Factor	New Effective Length	Polarity
7	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%	1.000164867	78.25289918	+
8	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%	1.000097569	78.24763379	+
9	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%	1.00004927	78.24385485	+
10	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%	1.000054724	78.24428161	+
11	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%	1.000070869	78.24554481	+
12	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%	1.000084129	78.24658226	+
13							1821.952	351.25								+
14							2121.933	383.03								+
15							2421.914	412.96								+
16							2721.895	441.21								+
17							112.986	108.23								-
18							301.998	140.04								-
19							601.979	180.77								-
20							1033.969	255.34								-
21							1333.969	284.76								-
22							1633.950	319.03								-
23							1933.931	352.55								-
24							2233.911	380.74								-
25							2533.892	412.99								-
26							2833.873	432.62								-
27											Averages:	0.04	0.02%	Averages:	78.24679942	

Figure S1d. "Stepped Field Reference Standards and Scale" spreadsheet

Step 4: Populate Columns A-K of the spreadsheet entitled "Stepped Field Data Format" with experimental data acquired in step 2 (see Fig. S1e).

- Columns L-Q will be auto-populated.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
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	Scaled CCS	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	=μ*(old effective length/ new effective length)^2	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	120.52	0.91
4	Your	Data	Here!														

Figure S1e. "Stepped Field Data Format" spreadsheet (Columns A-Q)

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

- Populate Columns R-W of the spreadsheet entitled "Stepped Field Data Format" (shown in Fig. S1f) with the classification information, source (e.g. research group), and DOI (if published).

	A	B	C	D	E	R	S	T	U	V	W
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 molecules	Fatty acyls	Fatty acids and conjugates	McLean	Unpublished
4	Your	Data	Here!			Your	Classifications	Here!			

Figure S1f. "Stepped Field Data Format" spreadsheet (Columns R-W)

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

- The CCS values submitted must meet the following criteria:
  - Data set RSD $\leq$ 0.5%
  - Individual RSD $\leq$ 0.7%

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

- Step 1: At minimum, triplicate measurements were acquired for reference standards and for each day that sample measurements were collected.
- Step 2: At minimum, triplicate measurements were 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_SteppedField_ScaleAndDataFormat.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