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.

- 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 1) to assess data quality.

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

$$\text{percent error} = \frac{(\text{CCS}_{\text{experimental}} - \text{CCS}_{\text{QA}})}{\text{CCS}_{\text{QA}}} \cdot 100$$

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

Step 3: Populate columns A-F in the spreadsheet entitled "Stepped Field Reference Standards and Scale" (see Fig. 1) 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 as well as the scaling procedure are discussed in supplemental Section S3.
- 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]
- The experimental effective length (in cm) needs to be entered in the yellow box (Cell D4) located at the top of the spreadsheet. This length can be found in the "BaseDataAccess.dll.config" file located in the Mass Hunter Workstation (Agilent Technologies) install directory (typically: C Drive > Program Files > Agilent > MassHunter > Workstation > IMS > B.07.02 > Bin). Alternately, this is the length value used in the initial CCS calculation that is to be scaled.
- Columns G-P 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***	***M/z* 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	152.95	152.95	152.78	322.048	153.73	322.05	1.04	152.89	0.10	0.07%	0.997278864	78.02709834	+
9	622.029	622.031	622.028	202.44	202.48	202.29	622.029	202.96	622.03	0.54	202.40	0.10	0.05%	0.998627769	78.13263665	+
10	922.010	922.012	922.009	242.09	242.42	242.34	922.010	243.64	922.01	0.36	242.28	0.17	0.07%	0.997213979	78.02202172	+
11	1221.991	1221.993	1221.990	280.82	280.84	280.77	1221.991	282.20	1221.99	0.27	280.81	0.03	0.01%	0.997533326	78.04700741	+
12	1521.971	1521.973	1521.970	316.34	316.40	316.42	1521.971	316.96	1521.97	0.22	316.39	0.04	0.01%	0.999095148	78.1692044	+
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:**	**0.08**	**0.04%**	**Averages:**	**78.10847795**	

Figure 1. "Stepped Field Reference Standards and Scale" spreadsheet

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

- CAS and/or InChi Key *must* be provided.
- Columns L-Q will be auto-populated.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	**Compound**	**Formula**	**CAS**	**InChi Key**	**Ion Species**	**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	JTERLNYVBOZRHI-RIIGGKAT	[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	Cyclosporin	C62H111N11O12		PMATZTZNYRCHOR-IMVLJIQESA-N	[M+H+K]	+2	620.9060	620.9068	620.9072	361.68	361.76	362.75	620.91	362.06	0.60	0.17	363.28	181.03
4	Cyclosporin	C62H111N11O12		PMATZTZNYRCHOR-IMVLJIQESA-N	[M+H+K]	+2	620.9060	620.9068	620.9072	373.66	373.09	372.45	620.91	373.07	0.60	0.16	374.32	186.53
5	Cyclosporin	C62H111N11O12		PMATZTZNYRCHOR-IMVLJIQESA-N	[M+H+K]	+2	620.9060	620.9068	620.9072	388.97	389.27	388.27	620.91	388.84	0.51	0.13	390.15	194.42
6	**Your**	**Data**	**Here!**															

Figure 2. “Stepped Field Data Format” spreadsheet (Columns A-R)

Step 5: Populate Columns S-T of the spreadsheet entitled “Stepped Field Data Format” (see Fig. 3) with experimental data acquired in step 2.

- Assign peak numbers in Column S: smallest CCS = 1, next smallest = 2, etc. If only one peak is observed, assign a “1”.
- Populate Columns T-U with the source (e.g. research group) and DOI (if published) information.

	A	B	C	D	E	F	R	S	T	U
1	**Compound**	**Formula**	**CAS**	**InChi Key**	**Ion Species**	**Charge**	**CCS/z**	**Peak Number**	**Source**	**DOI**
2	Example Lipid	C45H73NO8P	5634-86-6	JTERLNYVBOZRHI-RIIGGKAT	[M-H]	-1	277.30	1	Research Group	If unpublished, please fill in as "unpublished".
3	Cyclosporin	C62H111N11O12		PMATZTZNYRCHOR-IMVLJIQESA-N	[M+H+K]	+2	181.03	1	McLean	Unpublished
4	Cyclosporin	C62H111N11O12		PMATZTZNYRCHOR-IMVLJIQESA-N	[M+H+K]	+2	186.53	2	McLean	Unpublished
5	Cyclosporin	C62H111N11O12		PMATZTZNYRCHOR-IMVLJIQESA-N	[M+H+K]	+2	194.42	3	McLean	Unpublished
6	**Your**	**Data**	**Here!**							

Figure 3. "Stepped Field Data Format" spreadsheet (Columns R-X)

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 all experimental data set
  - Individual compound 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 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

Upon data submission, the data will temporarily be quarantined and a quality control assessment will be performed. The quality control assessment includes: (1) verifying that all inclusion criteria is met, (2) confirming that all pertinent information is provided, and (3) checking that data is formatted properly. After the authors have processed a dataset (typically less than 10 days), collaborators will be notified which values will be accepted or if any revisions are needed. Data will be made available as soon as the quality control assessment is complete.

Table 1. Quality Assessment (QA) Compound List

Standard reference CCS values obtained on a specially-modified drift tube instrument as previously reported.[2]

Compound	*m/z*	Ion Species	Stepped Field CCS (Å^2)	Single Field CCS (Å^2)
Small Molecules				
Cortisol	363.22	M+H	189.27 ± 0.10	188.34 ± 0.00
Cortisol	385.20	M+Na	213.72 ± 0.00	212.79 ± 0.07
Creatinine	112.05	M-H	120.69 ± 0.15	118.84 ± 0.07
Creatinine	114.07	M+H	123.86 ± 0.00	122.98 ± 0.02
Creatinine	136.05	M+Na	132.99 ± 0.35	132.61 ± 0.36
Glucose	203.05	M+Na	147.34 ± 0.29	146.94 ± 0.07
Homocysteine	136.04	M+H	130.77 ± 0.05	129.58 ± 0.63
L-arginine	173.10	M-H	138.03 ± 0.05	137.08 ± 0.01
L-arginine	175.12	M+H	136.84 ± 0.05	136.45 ± 0.00
L-aspartic acid	132.03	M-H	120.39 ± 0.40	119.15 ± 0.04
L-cystine	239.02	M-H	144.38 ± 0.09	143.58 ± 0.01
L-cystine	241.03	M+H	150.07 ± 0.05	149.48 ± 0.03
L-cystine	263.01	M+Na	151.81 ± 0.10	151.26 ± 0.13
L-glutamic acid	146.05	M-H	125.65 ± 0.15	124.47 ± 0.00
L-histidine	154.06	M-H	130.01 ± 0.09	128.83 ± 0.00
L-histidine	156.08	M+H	132.74 ± 0.11	131.93 ± 0.02
L-histidine	178.06	M+Na	135.47 ± 0.50	134.39 ± 0.44
L-isoleucine	130.09	M-H	131.28 ± 0.05	129.83 ± 0.01
L-isoleucine	132.10	M+H	133.81 ± 0.04	132.88 ± 0.03
L-leucine	130.09	M-H	132.51 ± 0.01	131.14 ± 0.00
L-leucine	132.10	M+H	135.55 ± 0.06	134.57 ± 0.03

Compound	*m/z*	Ion Species	Stepped Field CCS (Å²)	Single Field CCS (Å²)
Small Molecules (continued)				
L-lysine	147.11	M+H	131.62 ± 0.52	131.22 ± 0.14
L-methionine	150.06	M+H	134.07 ± 0.40	133.02 ± 0.47
L-phenylalanine	164.07	M-H	141.29 ± 0.19	139.94 ± 0.03
L-phenylalanine	166.09	M+H	141.27 ± 0.05	140.30 ± 0.12
L-proline	116.07	M+H	126.21 ± 0.20	125.38 ± 0.08
L-tyrosine	180.07	M-H	145.58 ± 0.34	144.42 ± 0.07
L-tyrosine	182.08	M+H	146.44 ± 0.20	145.58 ± 0.12
Levomefolic Acid	458.18	M-H	200.56 ± 0.11	198.99 ± 0.01
Levomefolic Acid	460.19	M+H	197.52 ± 0.26	197.17 ± 0.04
Pyridoxal Phosphate	246.02	M-H	150.80 ± 0.10	149.35 ± 0.04
Pyridoxal Phosphate	248.03	M+H	151.94 ± 0.10	151.37 ± 0.02
Pyridoxal Phosphate	270.01	M+Na	161.40 ± 0.20	161.46 ± 0.20
Uric Acid	167.02	M-H	126.92 ± 0.05	125.55 ± 0.07
Peptides				
Angiotensin1	1296.69	M+H	357.31 ± 0.26	355.62 ± 0.41
Angiotensin1	648.85	M+2H	387.29 ± 0.20	388.41 ± 0.10
Angiotensin1	432.90	M+3H	474.70 ± 0.15	477.05 ± 0.04
Angiotensin1	324.93	M+4H	549.23 ± 0.05	550.98 ± 0.07
Angiotensin2	1046.54	M+H	314.38 ± 0.15	313.65 ± 0.03
Angiotensin2	523.78	M+2H	353.79 ± 0.17	355.09 ± 0.03
Angiotensin2	349.52	M+3H	436.23 ± 0.20	437.30 ± 0.12
Bradykinin	1060.57	M+H	315.25 ± 0.30	314.00 ± 0.12
Bradykinin	530.79	M+2H	343.32 ± 0.10	344.99 ± 0.03
Bradykinin	354.19	M+3H	447.60 ± 0.11	449.07 ± 0.38
Melittin	1423.38	M+2H	613.36 ± 0.11	614.26 ± 0.02
Melittin	949.26	M+3H	721.06 ± 0.53	722.45 ± 0.02
Melittin	712.20	M+4H	756.78 ± 0.53	760.82 ± 0.12
Melittin	569.96	M+5H	808.60 ± 0.60	815.39 ± 0.10
Melittin	569.96	M+5H	844.39 ± 0.25	854.37 ± 0.15
Neurotensin	836.96	M+2H	434.32 ± 0.20	435.42 ± 0.06
Renin Substrate	879.97	M+2H	460.38 ± 0.40	461.11 ± 0.03
Renin Substrate	586.98	M+3H	518.81 ± 0.36	524.12 ± 0.07
Renin Substrate	440.49	M+4H	634.59 ± 0.35	637.65 ± 0.23
Substance P	1347.74	M+H	362.51 ± 0.20	361.44 ± 0.04
Substance P	674.37	M+2H	399.87 ± 0.20	400.09 ± 0.05
Substance P	449.92	M+3H	495.73 ± 1.29	496.51 ± 0.37
Proteins				
Cytochrome C	773.39	M+16H	3403.2 ± 2.10	3420.2 ± 2.38
Cytochrome C	727.96	M+17H	3538.1 ± 0.28	3554.7 ± 0.70
Cytochrome C	687.57	M+18H	3655.3 ± 1.57	3670.4 ± 0.74
Cytochrome C	651.44	M+19H	3741.8 ± 0.82	3757.9 ± 0.00
Cytochrome C	618.92	M+20H	3816.1 ± 0.79	3832.3 ± 0.00
Ubiquitin	856.98	M+10H	2192.3 ± 0.60	2204.8 ± 0.41
Ubiquitin	779.16	M+11H	2349.1 ± 0.77	2362.3 ± 0.00
Ubiquitin	714.32	M+12H	2424.6 ± 0.88	2444.2 ± 00
Ubiquitin	659.45	M+13H	2577.7 ± 0.63	2594.3 ± 0.53
Ubiquitin	612.41	M+14H	2727.4 ± 4.94	2728.8 ± 1.74
Ubiquitin	1223.80	M+7	1773.2 ± 1.26	1785.4 ± 0.29
Ubiquitin	1223.80	M+7	1875.7 ± 1.03	1884.3 ± 0.29
Ubiquitin	1070.96	M+8	1950.9 ± 0.24	1960.5 ± 0.33
Ubiquitin	952.08	M+9	2052.4 ± 0.64	2063.4 ± 0.00