## 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 1) 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%

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

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

Step 3: Populate columns A-F in the spreadsheet entitled "Single Field Reference Standards" (see Fig. 1) 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.
- Columns G-O 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 1.** "Single Field Reference Standards" spreadsheet

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

- CAS and/or InChi Key *must* be provided.
- Columns M-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 | InChi Key | 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 | JTERLNYVBOZRHI-RIIGGKATSA-N | [M-H] | -1 | 786.5070 | 786.5074 | 786.5109 | 277.30 | 277.33 | 277.27 | $\mu$ m/z | $\mu$ CCS | $\sigma$ | =$\sigma$/$\mu$CCS*100 | 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 | 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 | 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 | 194.42 |
| 6 | Your | Data | Here! | | | | | | | | | | | | | | |

**Figure 2.** "Single Field Data Format" spreadsheet (Columns A-L)

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

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

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| 2 | Example Lipid | C45H73NO8P | 5634-86-6 | JTERLNYVBOZRHI-RIIGGKATSA-N | [M-H] | -1 | 1 | Research Group | If unpublished, please fill in as "Unpublished".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| 3 | Cyclosporin | C62H111N11O12 | | PMATZTZNYRCHOR-IMVLJIQESA-N | [M+H+K] | +2 | 1 | McLean | Unpublished |
| 4 | Cyclosporin | C62H111N11O12 | | PMATZTZNYRCHOR-IMVLJIQESA-N | [M+H+K] | +2 | 2 | McLean | Unpublished |
| 5 | Cyclosporin | C62H111N11O12 | | PMATZTZNYRCHOR-IMVLJIQESA-N | [M+H+K] | +2 | 3 | McLean | Unpublished |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

**Figure 3.** “Single Field Data Format” spreadsheet (Columns R-W)

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: 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 RSDs.

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

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.

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**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 |
| 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 |
| **Compound** | ***m/z*** | **Ion Species** | **Stepped Field CCS (Å²)** | **Single Field CCS (Å²)** |
| ***Peptides (continued)*** | | | | |
| 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 |