FG3:

Publishing GC×GC data: What are we doing well and what can we improve?

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The metabolomics standards initiative (MSI)

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Proposed minimum reporting standards for chemical analysis
Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI)
The Multidimensional Standard Initiative

The 10th Multidimensional Workshop

Proposed minimum reporting standards for GC\times GC analysis

Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI)
Topic 1:

GC×GC Instrumental parameters
1) Injection parameters?
   1a) Hardware information
   1b) Software parameters
      - Liquid
      - HS (SPME, SHS, DHS, TD)
   Full description needed (not always the case...)
2) GC×GC parameters

2a) Hardware information
- Modulator, connections, ...
- Columns

2b) GC×GC parameters
- Oven temperature
- Flow, pressure or linear velocity?
- Modulator:
  - **Cryogenic**: Modulation time, $P_M$, hot/cold jet time?
  - **Flow**: differential vs diverting, auxiliary pressure, flow in the modulator, accumulation time and washing time? Split ratio?
  - **Phase ratio**: what to mention?
  - Modulator offset for ‘modified’ display of chromatograms? How to deal with wraparound?

• Full details to be made available
• Refer to previous papers
• Jet duration can be important
• Add all parameters for replication
• NO issue with wraparound
• All parameters MUST be listed
3) Detector

3a) Frequency of acquisition? [try to cover all the main detectors]

- Do we need to state the number of point for peak in average?
- Do we really need 200Hz?
- Deconvolution parameters: what we need to know?

- Frequency to be adapted... based on the needs... (sensitivity vs peak shape vs deconvolution...) to be clearly discussed
- Deconvolution is often ‘black box’, wrong settings lead to artefacts
- Guidelines to setup main parameters??
Topic 2:

Data processing
2a) Software parameters (Alignment parameters, S/N, Thresholds, etc)

2b) Post processing clean up: what needs to be done manually and what can be automatize? What do we need to report?

- Report all settings...
- Peak finding parameters (peak width, number of data points, changes over time)
- Report best fits
- Impossible to be exhaustive
- Detrect more/detect less?
- Make ‘raw’ data available... for re-processing from third parties...
• Should guidelines be published ??? Complicated
• Some processing parameters are not clear to users themselves (depending on the software)...
• Legacy software for later use ?
• How (where) to store data ? Related cost ?
• Data protection...
• Prove your point, no matter the path
• Be transparent
• ......
Topic 3:
Quality Control
3a) Tuning results and frequency?

3b) Necessity of (sample, instrumental, ...) blanks?

3c) Standards for instrument check: do we need it? How the QC-chart should be filled in? What parameters to be checked? What rules to be applied?

3d) Need for calculation of space occupation/orthogonality?

3e) Should experimental design parameters and plots be included?

- Rely on instrument tunes...
- Demonstrate control of hardware (QC charts on tRs, etc...) MANDATORY in forensics
- Space occupation not critical – results are driving methods
- Document blanks and report strategy
- Include QC samples
Topic 4:

Do we need a set of different minimum acceptable parameters to be reported according to the field of applications?
IGS™ Scoring Configuration

- Enable Similarity Check
- Minimum Similarity for Pass Rating (0 - 999): 800
- Minimum Valid Similarity (0 - 999): 700
- Enable Fragment Ion Check
- Minimum Abundance (100 - 998): 300
- Required Mass Accuracy: 5 +/- Mass Window
  - mDa
  - ppm
- Enable Molecular Ion Check
- Minimum Library Abundance (0 - 998): 100
- Required Mass Accuracy: 5 +/- Mass
  - mDa
  - ppm
- Enable Retention Index Check
- Retention Index Window: 25
4a) What minimum set of parameters for identification?

- $t_R^1$ (LRI, delta LRI ??)
- $t_R^2$ ?
- Library match value? (forward, reverse, probability, ...)
- Mass accuracy?
- Pure standard injections?
- ...

4b) What about initiatives like “Identification Grading System”?

- What default value to set?

- Difficult to define how confident we have to be
- Subjective viewpoints depending on reviewers/journals
- Need for a set of defined values
- Some proof of concept studies still needed
- Use of a ‘scoring system’
Topic 5:

Should we use the current ‘official nomenclature’?

Is it time for an updated nomenclature?

Do we need a nomenclature?

Column sets nomenclature to be used?
Nomenclature and Conventions in Comprehensive Multidimensional Chromatography

Nomenclature is very important
Users MUST use proper terms and layout
People to speak the same language
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modulator</td>
<td>Interface device between the two columns in a comprehensive two-dimensional separation system that accumulates or samples narrow bands from the eluate of the first column for fast re-injection into the second column.</td>
</tr>
<tr>
<td>Modulation time or Modulation period (P_m)</td>
<td>The duration of a complete cycle of modulation in a comprehensive two-dimensional separation system (equals the data conversion time of each second dimension chromatogram, i.e., the time between two successive injections into the second column).</td>
</tr>
<tr>
<td>Modulation frequency (f_m)</td>
<td>Number of modulations per unit of time.</td>
</tr>
<tr>
<td>Modulator temperature (T_m)</td>
<td>The temperature of the modulation zone used in thermal modulation.</td>
</tr>
<tr>
<td>Modulation number (n_m)</td>
<td>The number of modulated peaks recorded for a given first-dimension peak.</td>
</tr>
<tr>
<td>Modulation ratio (M_R)</td>
<td>The ratio of the peak width at baseline (W_R) for the first dimension peak to the modulation period (P_m).</td>
</tr>
<tr>
<td>Modulation phase ((\Phi; F_m))</td>
<td>The pattern of modulated peaks caused by the time relationship between the shape of the analyte peak and the pulsing process of the modulator in a comprehensive two-dimensional separation system (18).</td>
</tr>
<tr>
<td>In-phase modulation</td>
<td>The modulation phase that produces a symmetrical sequence of peaks with a single maximum peak pulse (18).</td>
</tr>
<tr>
<td>Out-of-phase modulation</td>
<td>Any phase that produces a non-symmetrical peak-pulse distribution (18).</td>
</tr>
<tr>
<td>180° out-of-phase modulation</td>
<td>The modulation phase that produces a symmetrical sequence of peaks with two equal maximum peak pulses (18).</td>
</tr>
<tr>
<td>Single-stage modulation</td>
<td>Accumulation and focusing during one series of processes at one location in the modulator.</td>
</tr>
<tr>
<td>Dual-stage modulation</td>
<td>Accumulation and focusing during two successive series of processes at two locations in the modulator.</td>
</tr>
<tr>
<td>Focusing effect</td>
<td>Reduction of the band width (in time, distance and/or volume units) (= band width without modulation/band width with modulation).</td>
</tr>
<tr>
<td>Sensitivity enhancement (= peak-amplitude enhancement)</td>
<td>Ratio between peak height with and without modulation (note: sensitivity refers to the signal, not to the noise!).</td>
</tr>
<tr>
<td>Zone compression</td>
<td>The effect of reducing a chromatographic peak (width) in space or time to give a higher concentration within a chromatography column.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Separation space</td>
<td>The region within the two-dimensional GC×GC plot in which compounds are, or may be, distributed.</td>
</tr>
<tr>
<td>Wrap-around</td>
<td>The occurrence of second dimension peaks in subsequent modulation sequences, caused by second-dimension retention times that exceed the modulation period of a comprehensive two-dimensional system (19).</td>
</tr>
<tr>
<td>Iso-volatility curves</td>
<td>The observation of reduced retention of a solute on a 1D column in GC×GC as the temperature of the oven increases, seen as a decreasing retention time ( t_R ), band in the 2D plot.</td>
</tr>
<tr>
<td>Column set</td>
<td>The combination of columns used for a given comprehensive 2D chromatography experiment.</td>
</tr>
<tr>
<td>Column set relative diameter ratio</td>
<td>The relative change in cross sectional area for the 1D to 2D columns of the column set = ( \frac{d_1}{d_c} ).</td>
</tr>
<tr>
<td>Chromatogram structure</td>
<td>The observed ordering of chemically related compounds in the plane of a comprehensive two-dimensional separation.</td>
</tr>
<tr>
<td>Colour plot</td>
<td>Two-dimensional plot representing a comprehensive two-dimensional separation, in which the colour represents the signal intensity of the components in the separation system.**</td>
</tr>
<tr>
<td>Contour plot</td>
<td>Two-dimensional plot representing a comprehensive two-dimensional separation, in which similar signal intensities of components are connected by means of a line.**</td>
</tr>
<tr>
<td>Apex plot</td>
<td>Two-dimensional plot representing a comprehensive two-dimensional separation, in which peak apexes of second-dimension peaks are displayed by a symbol in the 2D space. This may also be simplified to the peak apexes of individual components. **</td>
</tr>
<tr>
<td>Cryogenic/thermal modulation</td>
<td>GC×GC system in which the interface operates by changes in temperature compared with the oven temperature, either by setting an elevated or cooler temperature.</td>
</tr>
<tr>
<td>Diaphragm modulation</td>
<td>GC×GC system in which the interface operates by periodically selecting a small sub-fraction of the 1D peak to be transferred to the 2D column using a diaphragm system.</td>
</tr>
<tr>
<td>Flow modulation</td>
<td>GC×GC system in which the interface operates by a flow switching mechanism; normally a higher flow is maintained for the 2D column.</td>
</tr>
</tbody>
</table>

* A reduction in the detection limit may also be achieved. This reduction is proportional to the product of the sensitivity enhancement and the noise.  
** The x-axis represents the first-dimension retention time, the y-axis the second-dimension retention time of the separation system.
<table>
<thead>
<tr>
<th>Symbols</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1d_1, 1d_2</td>
<td>Internal diameters of the first- and second-dimension columns (respectively) used in a comprehensive two-dimensional system.</td>
</tr>
<tr>
<td>1D, 2D</td>
<td>First dimension and second dimension of a C2DC system</td>
</tr>
<tr>
<td>1D, 2D</td>
<td>One dimensional or two-dimensional system</td>
</tr>
<tr>
<td>1t_1, 1t_2</td>
<td>Retention times of a peak in the first and second dimension of a comprehensive two-dimensional system (respectively). Note that 1t can potentially differ for each modulated peak of a given injected component.</td>
</tr>
<tr>
<td>1t_n, 1t_m</td>
<td>Hold-up times (or “dead” times) of the first and second columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1k, 1k</td>
<td>Retention factors of a peak eluting from the first-and second-dimension columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1l, 1l</td>
<td>Retention indices of a peak eluting from the first- and second-dimension columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1N, 1N</td>
<td>The numbers of theoretical plates of the first and second columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1N_w, 1N_m</td>
<td>The numbers of effective plates of the first and second columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1σ, 1σ</td>
<td>Standard deviations of a peak eluting from the first-and second-dimension columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1W_w, 1W_m</td>
<td>Peak widths at base of a peak eluting from the first-and second-dimension columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1R_e, 1R_s</td>
<td>Resolution values of a peak pair eluting from the first and second column of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1n_e, 1n_s</td>
<td>Peak capacities of the first and second columns of a comprehensive two-dimensional system (respectively) [the use of n_i is advised to avoid confusion with n that is sometimes used for theoretical plates]</td>
</tr>
<tr>
<td>1d_1, 1d_2</td>
<td>Film thicknesses of the first and second columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1μ, 1μ</td>
<td>Average linear velocities in the first and second columns of a comprehensive two-dimensional system (respectively).</td>
</tr>
<tr>
<td>1T_e, 1T_s</td>
<td>Elution temperatures for a peak eluting from the first dimension and second dimension of a comprehensive two-dimensional GC system (respectively). (Note that 1T_e and 1T_s will be essentially the same due to the very fast elution of components on 1D for the GC×GC experiment, defining isothermal elution)</td>
</tr>
<tr>
<td>1T_m</td>
<td>Modulator temperature</td>
</tr>
<tr>
<td>1P_m</td>
<td>Modulation period</td>
</tr>
<tr>
<td>1M</td>
<td>Modulation ratio</td>
</tr>
<tr>
<td>1t_1</td>
<td>Apparent first dimension retention time of the component on the first dimension</td>
</tr>
<tr>
<td>1t_n</td>
<td>Hold time of the peak in the modulator</td>
</tr>
<tr>
<td>1A_s</td>
<td>Two-dimensional peak asymmetry</td>
</tr>
</tbody>
</table>
Topic 6:

Do we need to always show at least one chromatogram?

What about apex plots vs real peaks?

Pseudochromatograms? FR ‘bubble’ plots?
• Apex plots are an efficient way to report chromatographic data
• Always support findings with chromatograms
• ‘Bubble plots’ efficient to localize relevant analytes
• Bubbles can hide other analytes if too big...
• Use a mix of displays to illustrate and support findings
Topic 7:

What should Supplementary Materials typically made of?

How detailed should they be?

Should raw data be made available?
Untargeted Blood Metabolic Profiling by GC×GC-HRTOF-MS

Supporting Information

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S-6. Injection sequence of the Crohn’s disease study.
S-8. QC system. LOESS procedure.
S-14. Regression methods and LOD/LOQ assessment. A. Determination of the best fit of LOD and LOQ.
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S-17. Statistics for biomarker research.
S-18. Data Control and Selection Process for the three Crohn’s disease groups candidates.
S-19. Data Control and Selection Process the three Crohn’s disease groups candidates.
S-23. Testing of potential bias between training and test sets for all samples.
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Topic 8:

What else?
Thank You