MIRAGE LC Guidelines

Guidelines for reporting liquid chromatography experiments

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Classification	Definition
1. General Features, Global MIRAGE Descriptors	
Responsible person or institution - provide name, affiliation and contact information	The primary contact person for this data set; this could be the experimentalist, principal investigator etc. Provide supporting information including Department, Institution, Email and Phone number (not compulsory). Additional persistent digital identifiers e.g. ORCID can be used.
Time stamp	Date and time the LC experiment was performed (start time).
Sample Preparation	Any general and specific parameters, settings, conditions important for tracing the sample preparation history as outlined in the sample preparation guidelines. The MIRAGE Sample Preparation guidelines (doi:10.3762/mirage.1) include all aspects of sample generation, purification and modification from biological and/or synthetic carbohydrate material.
2. Equipment	
A wide variety of LC instrumentation can be used to analyze complex samples in various glycomics approaches. As the utility of the data generated from a LC analysis will be related to the type of chromatography and its configuration minimal information should include:	
Manufacturer	The name of the manufacturer for a combined unit or component parts including operating software version and firmware.
Model	The model name provided by the manufacturer for the system and/or component parts.
Instrument details	Type of tubing used (fused silica, steel), including inner diameter and length (in particular for LC-MS), sample loop material, extra column volumes, any custom modifications. (e.g. precolumn heater,…).
2.1 Column Details & Characteristics	
Manufacturer	The name of the manufacturer.
Model	The model number / batch number provided by the manufacturer.

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Separation Mode	A description of the type of column used, therefore, the separation mechanism: e.g. normal phase, reversed phase, weak anion/cation exchange, PGC,…
Column Dimensions	The dimensions of the column in terms of length and inner diameter, if relevant the total volume of the column.
Stationary Phase	A description of the constituents of the stationary phase, including the name of the packing material and the particle size, or describe the packing materials in the case of monoliths. Provide manufacturer/supplier details if the stationary phase was obtained separately from column i.e. in-house column packing. In case of in-house packing, provide details on the packing conditions/instruments used.
Column Heater	Description of temperature range.
Additional accessories	Details of any guards, traps, pre-columns or inline filters used in conjunction with the column.
2.2 Mobile Phase	
Description of solvent	Name used to refer to each mobile phase that is further explained in the *Properties of Column Run* section. Prop. Solvents description.
Description of Constituents	For each constituent (solvents and additives), name of supplier, a description of grade/quality/purity, the concentration, pH (and how adjusted).
2.3 Properties of the Chromatographic Run (parameters that may change over run time)	
Time	The total time of the column run with appropriate units including pre-runtime, runtime and post-runtime.
Gradient	The percentage of each mobile phase, relative to time, including its overall duration.
Flow Rate	The flow rate at which the mobile phase is applied to the column, including the time period for which this is true.
Temperature	The temperature at which the column is run and any time points this varies; any other temperature sensitive aspects (e.g. temp of an optional precolumn heater,…).
Sample Injection	A description of the injection procedure, such as details of the volume, buffer, inline/offline, direct/loop (full/partial loop), flush conditions, and sample storage temperature, sample loop material, type of sample vials used.

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2.4 Pre- and Post Run Processes	
Type	A description of the purpose of the process, such as equilibration or washing (this may be part of the column run, as one step or as a preconditioning of the column prior to use).
Substance, Standards	A description of the reagent used in the process. For example, this can be applied to e.g. dextran or arabinose ladders for establishing a retention index (i.e. a normalization of retention times). Addition of internal standards of known structure and amount (to support inferred quantitation).
Time	The duration of the pre- and post run process.
Flow Rate	The rate at which the mobile phase is applied to the column. Type of gradient mixer.
Post separation events	Post column derivatization(s) and additives.
Column Regeneration	Description of column regeneration, approx. number of injections on to column until separation deteriorates.
Equipment used for detection	Manufacturer and model, or description.
Type	A description of the kind of detector (e.g. UV, Fluorescence, MS). In case of MS detection, refer to the MIRAGE MS guidelines (doi:10.3762/mirage.2) for specific parameter descriptions.
Detection system	All parameters with the potential to influence peak height, peak width, detection,… e.g. flow cell volume and path length - if peak detection is achieved by mass spectrometry, please also refer to MS reporting guidelines.
Equipment Settings	A description of control properties of the detector, such as the wavelength(s), gain, frequency that is being detected, sampling frequency (e.g. 10 Hz).
Timescale over which data was collected	The time range covered by the trace produced by the detector.
2.5 Column outputs – Fractions (if separation purpose is preparative)	
Fraction name	An optional name, unique within a run, by which a fraction can be referenced.
Fraction description	Either a description of the procedure by which the fractions were collected i.e. start/end time, mode (fixed or peak directed), or a description of the individual fractions

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	(e.g. time of collection, volume, charge).
Flow split	Splitter type and split ratio
LC-MALDI Spotting	Describe all instruments and parameters specific for target spotting (e.g. spot size, fractionation time, matrix, flowrate - for subsequent MALDI detection please refer to MS reporting guidelines).
2.3 Data annotation	
Database	Specify database used to assign structures based on retention index (name, version).
Software	Specify any software used to assist data interpretation (name, version). If open source software is used include web site or download link for re-evaluation of data and results.
Peak selection	Define peak criteria including if manually or automatically integrated, integration parameters.
Peak quantitation	Please provide chromatogram trace processing parameters such as smoothing etc.
Trace output	Provide (at least) the profile trace i.e. sample not treated with enzymes. The location (URL or database identifier) and format of the trace if appropriate. Additional data may include exoglycosidase trace(s).
3. Exoglycosidase Treatment	
Sequencing oligosaccharides by exoglycosidases, either sequentially or in an array format, is a powerful tool to unambiguously determine the structure of complex glycans. General information on the preparation of exoglycosidases should include:	
Supplier	Name of supplier
Exoglycosidase preparation (ref to sample prep)	Volume and concentration (also of sample), (any deviation from manufactures recommendations). Provide the units of enzyme added to the sample based on the manufactures unit definition.
Reaction time (ref to sample prep)	Total reaction time (mins) this may reflect expected behaviour (partial or complete digestion).

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Control	Describe any external controls used to monitor enzyme activity, include the data of these controls, provide details if digestion protocols were adjusted on the basis of the controls.
Protocol	Link to published/suitable exoglycosidase protocol.

Example LC Data Annotation

Results of LC analyses are generally summarized in table form showing the relative abundance and retention time of all characterized glycans (Peak Number). Supporting LC profiles are provided in text or as supporting material. Regardless of the data presentation format the minimal information required should include peak number, standardized retention time, structure, peak area, and if confirmed by exoglycosidase treatment. Additional fields may include the use of weak anion exchange chromatography or similar for charge confirmation. For LC data assigned without supporting exoglycosidase digestions or complementary mass spectrometry analysis, we recommend reporting all (potential) structures co-eluting within a specified time/GU range.

Proposed Data Summary

Peak #	Structure	Public Database ID	Retention Time (UND) In GU/AU	Peak Area	Exoglycosidase Treatment
Peak number	Image	GlyTouCan Id (or similar)	Retention	Percentage peak area	Description of exoglycosidases used to confirm structure