# MIAPE: Column Chromatography

Andrew R Jones[1,†], Kathleen Carroll[2], David Knight[3], Kirsty MacLellan[4], Paula J Domann[5], Cristina Legido-Quigley[6], Lihua Huang[7], Lance Smallshaw[8] and Norman W Paton[9]

[1]Department of Pre-clinical Veterinary Science, Faculty of Veterinary Science, The University of Liverpool, Liverpool, L69 7ZJ, UK.
[2]Manchester Centre for Integrative Systems Biology, Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess Street, Manchester, M1 7DN, UK.
[3]Faculty of Life Sciences, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK.
[4]IMPMC, Université Pierre & Marie Curie, Campus BOUCICAUT 140, rue de Lourmel, 75015 Paris, France.
[5]LGC Ltd, Teddington, Middlesex, TW11 0LY, UK
[6]PSD, King's Colllege London, SE1 9NH, UK.
[7] Bioproduct Research and Development, Lilly Research Laboratories, Lilly Technology Centre, Indianapolis, Indiana, USA.
[8]Lilly UK, Speke, Liverpool, UK.
[9]School of Computer Science, University of Manchester, Oxford Road, Manchester, M13 9PL, UK.

† Corresponding author, email: andrew.jones@liv.ac.uk

**Abstract**

MIAPE - Column Chromatography (MIAPE-CC) is one module of the Minimal Information About a Proteomics Experiment (MIAPE) documentation system. MIAPE is developed by the Proteomics Standards Initiative of the Human Proteome Organisation (HUPO-PSI). It aims at delivering a set of technical guidelines representing the minimal information required to report and sufficiently support assessment and interpretation of a proteomics experiment. This MIAPE-CC module is the result of work carried out through the Sample Processing Workgroup of the Proteome Standards Initiative. It has been designed to specify a minimal set of information to document a column chromatography experiment.

# MIAPE: Column Chromatography

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**This module identifies the minimum information required to report the use of columns in a proteomics experiment, in a manner compliant with the aims as laid out in the 'MIAPE Principles' document [1]**

## Introduction

Several techniques exist within the proteomics domain to facilitate the separation of mixtures. One such approach involves the use of columns, which can be used to separate proteins or peptides based on a range of physical or chemical properties. For example, ion exchange chromatography separates on the basis of surface charge, and affinity chromatography separates based on ligand affinity. These reporting guidelines cover the use of columns for protein or peptide separation, with a view to supporting the sharing of best practice, validation of results, discovery of results and sharing of experimental data sets. For a full discussion of the principles underlying this specification, please refer to the MIAPE 'Principles' document [1].

These guidelines cover a column chromatography experiment from the selection and configuration of a column, through the selection of a suitable mobile phase and verification of the relevant performance characteristics, to the collection of fractions and associated detector readings. These guidelines do not explicitly cover a sample preparation procedure but facilitate the description of the sample. They do not include protein identification procedures. Where multidimensional chromatography is used, the material below is repeated as required for each dimension, with specific fractions from one column being used as the sample for another. Items falling outside the scope of this module may be captured in complementary modules, which can be obtained from the Proteomics Standards Initiative's website (http://www.psidev.info/index.php?q=node/91).

Note that subsequent versions of this document may have altered scope, as will almost certainly be the case for all the MIAPE modules.

The following section, detailing the reporting requirements for column chromatography is subdivided as follows:

1. General features
2. Equipment
3. Mobile Phase
4. Properties of the Column Run
5. Pre and Post Run Processes
6. Column Outputs

If multiple dimensions of separation are performed, Sections 2-6 should be repeated as appropriate.

## Reporting requirement for Column Chromatography

**1.** ***General features***

1.1. Global Descriptors
  1.1.1. Date stamp (as YYYY-MM-DD)
  1.1.2. Responsible person (or institutional role if more appropriate); name, affiliation and stable contact information

1.2. Sample
  1.2.1. Brief description of sample
  1.2.2. Processing applied to the sample
  1.2.3. Sample injection

**2.** ***Equipment***

2.1. Product Details for Column
  2.1.1. Manufacturer
  2.1.2. Model
  2.1.3. Separation mode

2.2. Physical Characteristics of Column
  2.2.1. Dimensions
  2.2.2. Description of stationary phase
  2.2.3. Additional accessories

2.3. Chromatography System used for the Separation
  2.3.1. Manufacturer
  2.3.2. Model

**3.** ***Mobile Phase:*** for each mobile phase

3.1. Name of mobile phase

3.2. Description of constituents

4. ***Properties of the Column Run***
   - 4.1. Time
   - 4.2. Gradient
   - 4.3. Flow rate
   - 4.4. Temperature

5. ***Pre and Post Run Processes***
   - 5.1. Type
   - 5.2. Substance
   - 5.3. Time
   - 5.4. Flow Rate

6. ***Column Outputs***
   - 6.1. Detection
     - 6.1.1. Equipment used for detection
     - 6.1.2. Type
     - 6.1.3. Equipment settings
     - 6.1.4. Timescale over which data was collected
     - 6.1.5. Trace
   - 6.2. Fractions
     - 6.2.1. Fraction name
     - 6.2.2. Fraction description

In multi-dimensional separations, stages 1(b) to 6 are repeated.

## Summary

The *MIAPE: Column Chromatography* minimum reporting requirements for columns for protein separation specify that a significant degree of detail be captured, for separations according to different physical and chemical criteria. However, providing the information required by this document should enable the effective interpretation and assessment of the results of downstream analyses, for example to identify or quantify the proteins present in a sample and potentially, support experimental corroboration. Much of the information required herein may already be stored in an electronic format, or exportable from the instrument; we anticipate further automation of this process.

## References


1. Taylor C.F., Paton N.W., Lilley K.S., Binz P.-A., Julian R.K. Jr, Jones A.R., Zhu W., Apweiler R., Aebersold R., Deutsch E.W., Dunn M.J., Heck A.J.R., Leitner A., Macht M., Mann M., Martens L., Neubert T.A., Patterson S.D., Ping P., Seymour S.L., Souda P., Tsugita A., Vandekerckhove J., Vondriska T.W., Whitelegge J.P., Wilkins M.R., Xenarios I., Yates III J.R., Hermjakob H., The minimum information about a proteomics experiment (MIAPE), *Nature Biotech*, August;25(8), 887 - 893, 2007. http://www.nature.com/nbt/journal/v25/n8/abs/nbt1329.html.

**Appendix One.** The MIAPE: Column Chromatography glossary of required items

| *Classification* | *Definition* |
|---|---|
| *1. General Features* | |
| *1.1. General Features – Global Descriptors* | |
| 1.1.1. Date Stamp | The date on which the work described was initiated; given in the standard 'YYYY-MM-DD' format (with hyphens). |
| 1.1.2. Responsible person or role (or institutional role if more appropriate); provide name, affiliation and stable contact information | The (stable) primary contact person for this data set; this could be the experimenter, lab head, line manager, etc. Where responsibility rests with an institutional role (e.g. one of a number of duty officers) rather than a person, give the official name of the role rather than any one person. In all cases give affiliation and stable contact information. |
| *1.2. General Features – Sample* | |
| 1.2.1. Brief description of sample | A description of the source, such as means of collection, volume, concentration or previous step of processing. Include information about tags, molecular mass, pH and anything else relevant to experiment. In a multi-dimensional separation, this should contain the name of the fraction from the previous dimension. |
| 1.2.2. Processing applied to the sample | A brief description of processing applied to the sample, including the buffer, the volume, any pre-treatments (such as the addition of an acid to facilitate binding) and any tag applied e.g. for fluorescence detection. |
| 1.2.3. Sample injection | A description of sample injection procedure, such as details of the volume, inline/offline, direct/loop (full/partial loop), flush conditions, and sample storage temperature. |
| *2. Equipment.* | |
| *2.1. Equipment. – Product Details for Column* | |
| 2.1.1. Manufacturer | The name of the manufacturer. |
| 2.1.2. Model | The model number provided by the manufacturer. |
| 2.1.3. Separation mode | A description of the type of column being used. (e.g., Separation mechanism: Affinity, Anion Exchange, Cation Exchange, Reverse Phase, Hydrodynamic Volume). |
| *2.2. Equipment. – Physical Characteristics of Column* | |
| 2.2.1. Dimensions | The dimensions of the column in terms of length and inner diameter or the total volume of the column where this value is appropriate, for example to describe gel filtration experiments. |
| 2.2.2. Description of stationary phase. | A description of the constituents of the stationary phase, including the name of the packing material and the particle size, or describe packing materials in the case of monoliths including their manufacturer if stationary phase obtained separately from column. |
| 2.2.3. Additional accessories | Details of any guards or traps used in conjunction with the column. |
| *2.3. Equipment. – Chromatography system used for the separation, where applicable.* | |
| 2.3.1. Manufacturer | The name of the manufacturer of combined unit or component parts including software used to operate |
| 2.3.2. Model | The model name provided by the manufacturer. |

| | |
|---|---|
| *3. Mobile Phase: for each mobile phase* | |
| 3.1. Name of mobile phase | Name used to refer to mobile phase in *Properties of Column Run*. |
| 3.2. Description of Constituents | For each constituent, a description, the concentration, pH (and how adjusted) and date prepared |
| *4. Properties of the Column Run (parameters that may change over time)* | |
| 4.1. Time | The total time of the Column Run with appropriate units. |
| 4.2. Gradient | The proportion of each of the mobile phases, relative to time , for the function describing the gradient, including its overall duration. There may be several steps that together make up the gradient. |
| 4.3. Flow rate | The flow rate at which the mobile phase is applied to the column, including the time period for which this holds if it varies during the experiment. |
| 4.4. Temperature | The temperature at which the column is run, including the time period for which this holds if it varies during the experiment. |
| *5. Pre and Post Run Processes.* | |
| 5.1. Type | A description of the purpose of the process, such as equilibration, calibration or washing (this may be part of the column run, as one step or as a preconditioning of the column prior to use). |
| 5.2. Substance | A description of the reagent used in the process. |
| 5.3. Time | The duration of the process. |
| 5.4. Flow rate | The rate at which the mobile phase is applied to the column. |
| *6. Column outputs* | |
| *6.1. Column outputs – Detection (if appropriate – this section would not normally be used for post-processing such mass spectrometry, for which additional MIAPE modules exist. In addition, this section may not be required if the trace has not been used for an analytical purpose.)* | |
| 6.1.1. Equipment used for detection | Manufacturer and model, or description. |
| 6.1.2. Type | A description of the kind of detector (e.g. UV, MS). |
| 6.1.3. Equipment settings | A description of control properties of the detector, such as the wavelength that is being detected. |
| 6.1.4. Timescale over which data was collected | The time range covered by the trace produced by the detector. |
| 6.1.5. Trace | The location and format of the trace if appropriate. |
| *6.2. Column outputs – Fractions (if separation purpose is preparative)* | |
| 6.2.1. Fraction name | An optional name, unique within a run, by which a fraction can be referenced. |
| 6.2.2. Fraction description | Either a description of the procedure by which the fractions were collected i.e. start/end time, size (time or volume), mode (fixed or peak directed), or a description of the individual fractions (e.g. time of collection, volume). |