Application Note

Tryptic Mapping Automated on the CyBi®-RoboSpense and Integration of an Agilent 1100/1200 HPLC System for Peptide Analysis

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Peptide mapping is an important analytical technique widely used to study the primary structure of proteins. In quality control settings, peptide mapping is employed as an identity test to probe for small changes in protein primary structure. Long incubation times and time consuming purification steps characterize this method. The complete manual preparation process takes about eight hours occupying one technician a whole day. Therefore, automation of the process is desired. An additional benefit of automation is data continuity and reproducibility.
The following application note describes automation of the tryptic mapping process on the CyBi®-RoboSpense liquid handling platform. Integrating an Agilent 1100/1200 HPLC system completes the automation. Thus, any manual interactions from reading sample tube barcodes to generating peak profiles by HPLC are eliminated.

Fig 1: CyBi®-RoboSpense platform for flexible liquid handling with an integrated Agilent 1100/1200 HPLC system

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Application Note

Integrated modules needed for this application:

- barcode reader for automatic reading of single tubes during loading
- cooled positions for tubes and plates (including deep well plates)
- vacuum station with adapter for purification columns
- thermocycler for high temperature incubation
- Automation Interface G2254A (Agilent)

Fig 2: Automation Interface Agilent Technologies

METHODS

During the mapping of proteins a series of incubations and buffer changes are required. To automate these steps switching from classic dialysis using membranes to column based methods has become necessary. The peptide mapping procedure uses columns originally made for nucleic acid purification to change buffers between the different protein digestion steps. This results in a faster and automated procedure for buffer exchange.

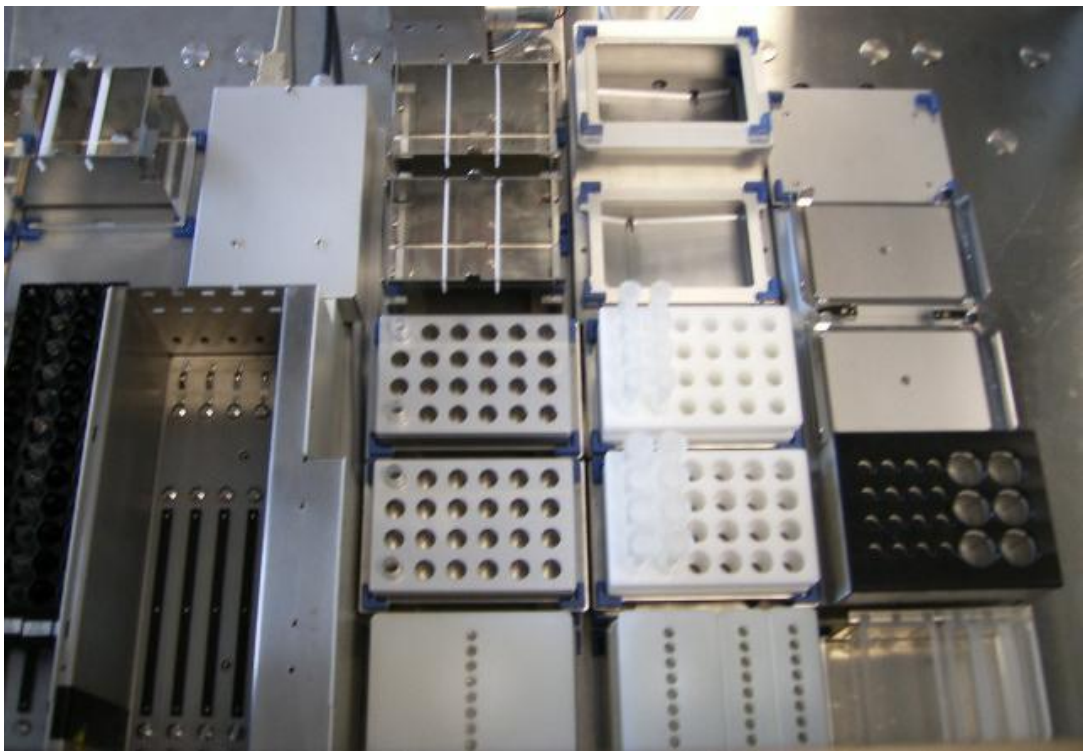

Fig 3: CyBi®-RoboSpense deck layout for peptide mapping

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Tryptic mapping procedure:

1. Samples are loaded onto the CyBi®-RoboSpense with positive sample identification using an integrated barcode scanner for single tubes. Missing barcodes or samples will be indicated immediately.
2. Concentrations of barcode identified samples are imported from a file and the samples are automatically diluted to the desired concentration.
3. The normalized samples are transferred into purification columns and eluted with the buffer needed for the next incubation steps.
4. Reagent A is added to all samples. After several minutes of incubation at ambient temperature reagent B is added followed by an additional incubation step.
5. After this incubation is completed samples are loaded into buffer exchange columns.
6. All steps during the buffer change and the elution of samples to fresh reagent vessels are performed on the drain position of the vacuum station.
7. Enzyme is added for digestion and samples are incubated several hours at ambient temperature.
8. Digestion was stopped by either a stop reagent or a high temperature step (at 95°C).
9. Finally, samples are transferred to the Automation Interface G2254A of the HPLC system connecting both instruments.

RESULTS

Parallel and subsequent mappings of the same protein sample (data not shown) as well as a comparison of the manual and the automated procedure (Fig. 4) showed very good reproducibility. The displayed chromatograms were generated with an Agilent 1100 HPLC system connected directly to the CyBi®-RoboSpense liquid handling robot.

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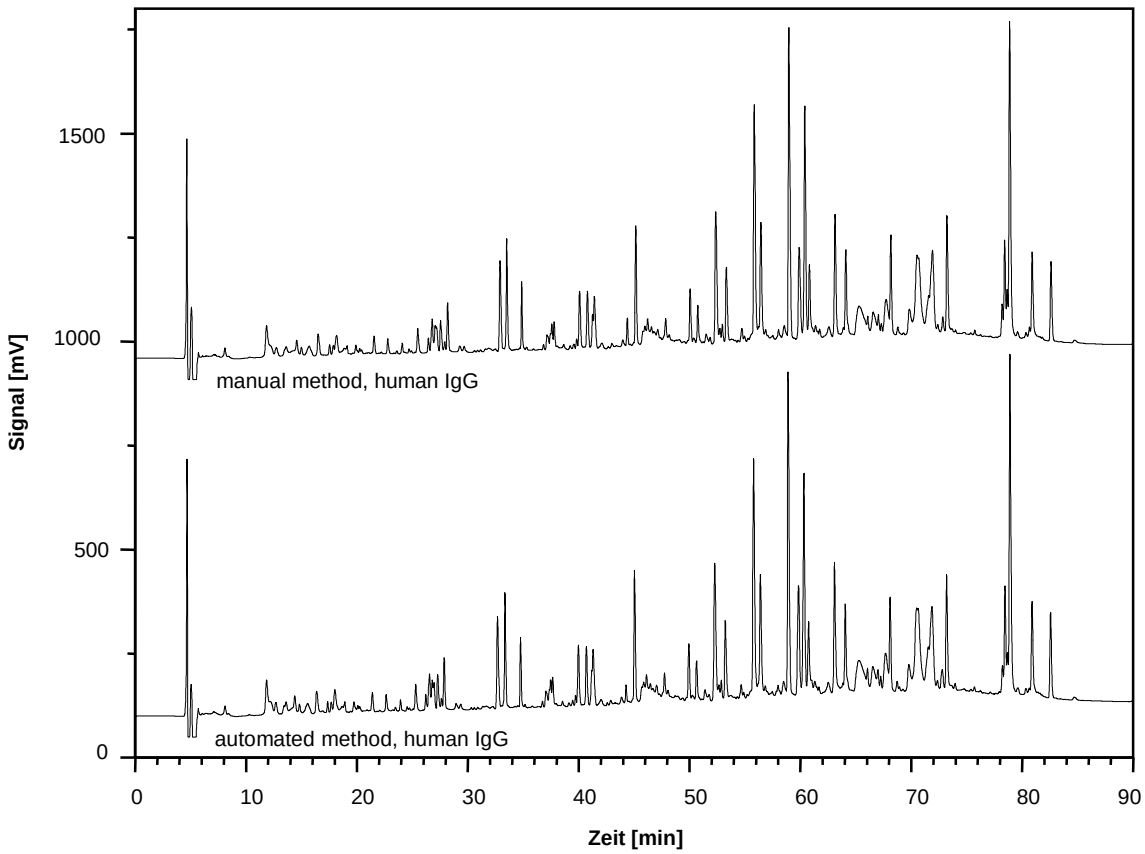

Fig 4: Comparison of manual and automated processing of the same protein analyzed with an Agilent HPLC system.

CONCLUSIONS

- Peptide mapping can be automated by substitution of dialysis with a column based exchange of buffers.
- The CyBi®-RoboSpense liquid handling system automates the complete routine from isolation of proteins to final HPLC results eliminating process bottlenecks.
- Due to the column based design the process is scalable from 1 to 24 samples without loss of buffer or consumables.
- Automation of peptide mapping with CyBi®-RoboSpense reduces human errors resulting in improved data quality.

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