DEVELOPMENT OF AN ANALYSIS METHOD TO ASSESS THE OCCUPATIONAL RISK DEALING WITH THERAPEUTIC MONOCLONAL ANTIBODIES USING LIQUID CHROMATOGRAPHY AND HIGH-RESOLUTION MASS SPECTROMETRY (LC-HRMS)

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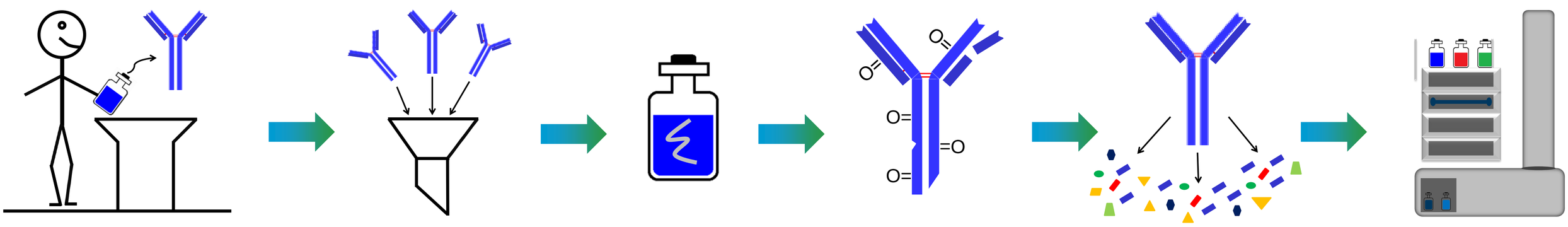
Introduction

- In an ongoing debate about the health risk assessment of monoclonal antibodies (mAbs) the following points need to be considered:
  - Long-term low dose data is not available
  - Pulmonary uptake of lyophilized powder or mAbs in aerosols is the most probable route of entry
  - Risk of sensitization outgoing from long-term low doses cannot be excluded
  - Safety recommendation varies greatly, from protective gloves alone to full protective clothing
- We present the development of an analytical method that enables us to determine the occupational exposure
  - Intact mAbs, heavy chain, light chain or peptides were investigated as suitable starting points
  - Analysis of intact or reduced mAbs leads to a large, sensitivity decreasing charge pattern
  - Peptide based workflows need extensive sample preparation, which can induce chemical modifications
  - Peptide level offers the opportunity to use a sum parameter for all mAbs
  - Expected occupational exposition is in the range of several µg/working shift

Conclusion and Outlook

- Development of a peptide based analytical workflow to quantify monoclonal antibodies (mAbs) as single substances as well as sum parameter is shown
- Limit of detections between 1 µg/sample for daratumumab and 25 µg/sample for the sum parameter were reached
- Stability of peptides in 24 h long-term air sampling was proved
- Recovery rates between 80% and 120% were observed
- Future investigations will be performed to collect experimental data of the occupational exposure of pharmacy staff to airborne mAbs
  - The resulting data will help to build a solid base for further discussions about the occupational health

Analytical Workflow



Results and Discussion

Intact monoclonal antibodies

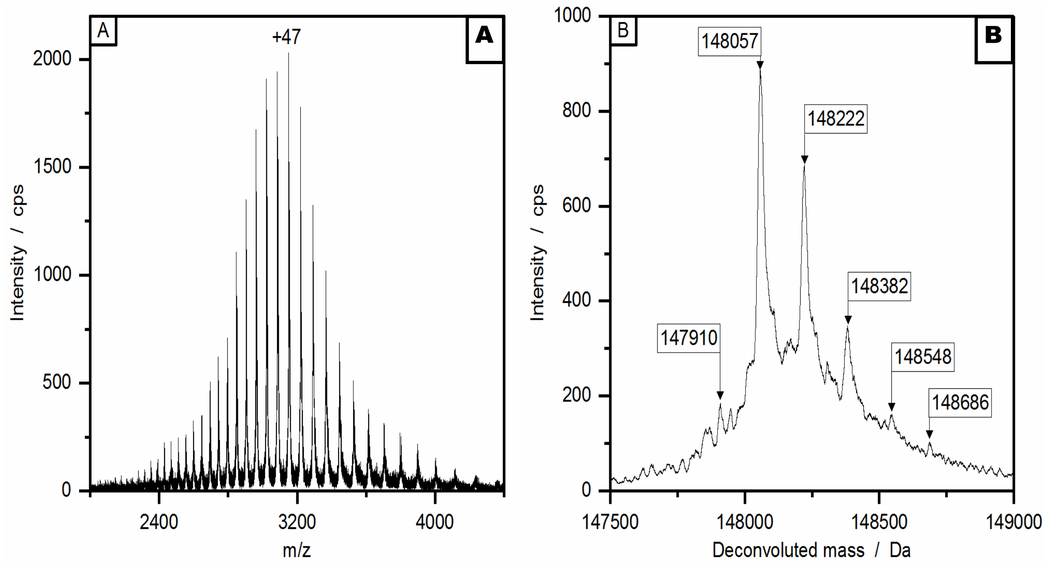


Figure 1: A: Mass-to-charge distribution of trastuzumab at denaturizing conditions by LC-ESI-MS with z = +47 as most intensive signal. B: The deconvolved masses are about 148 kDa depending on their glycosylation profile.

- LC-ESI-MS of monoclonal antibodies leads to a large charge pattern
  - Calculation of the MW with a deconvolving algorithm such as MaxEnt
- Intensity distribution over the single charge states leads to a sensitivity loss
- Peptide level offers higher sensitivity, due to less charge distribution

Air sampling

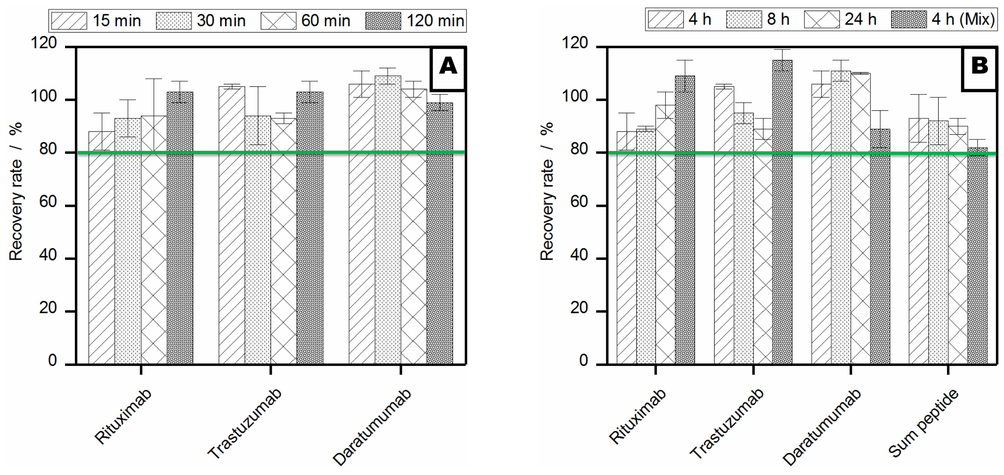


Figure 3: A: PVDF-filter were spiked with monoclonal antibodies solutions and extracted using pH 7.4 phosphate buffered saline (PBS) at 30 ° C and varying extraction time. B: The spiked PVDF-filter were connected to a membrane pump with a constant airflow of 2 L/min and varying sampling time. The extraction time was constant at 15 min.

- Extraction times of 15 min is sufficient
- Long-term sampling up to 24 h is possible
- No negative interaction between the monoclonal antibodies in a mix

Identification of stable peptides

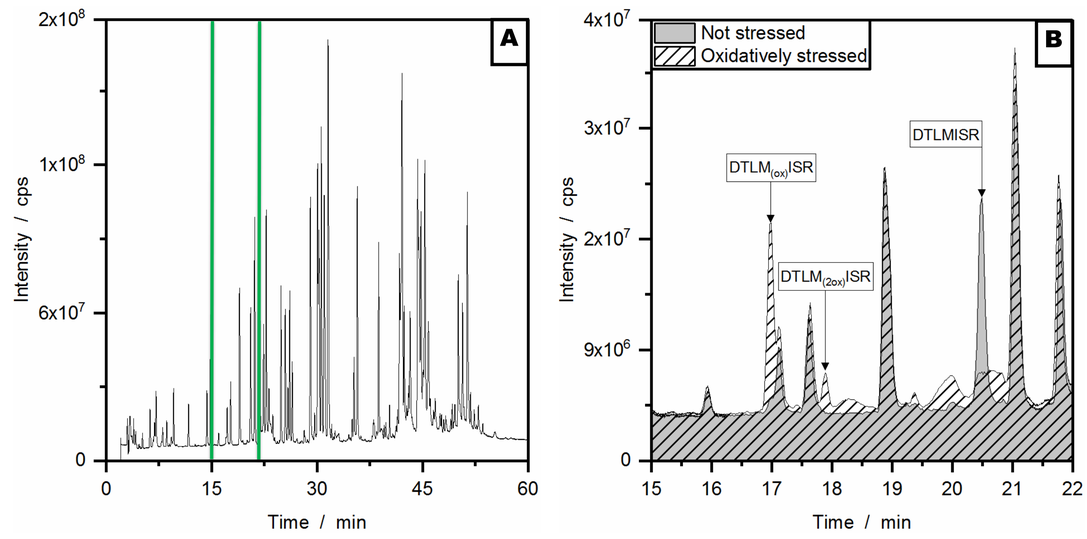


Figure 2: A: Chromatogram of a tryptic digest of trastuzumab. B: Enlargement from 15 min to 22 min. The effects of the oxidising agent H_2O_2 (lined) on trastuzumab are compared with an NaCl solution (grey) treated sample. The H_2O_2 treated sample was incubated. The NaCl solution was adjusted to physiological conditions.

- Tryptic digests of monoclonal antibodies lead to a large number of peptides
  - Only one signature peptide for the single substances and one collective peptide to create a sum parameter is necessary
- Instable peptides are not suitable for an analytical workflow
  - Forced degradation tests with an acidic, a basic and an oxidative medium
- Stable and chosen peptides
  - Rituximab FSGSGSGTSYSLTISR (*m/z* 803.8890; z = 2)
  - Trastuzumab LLIYSASFLYSGVPSR (*m/z* 886.9827; z = 2)
  - Daratumumab LLIYDASNR (*m/z* 532.7904; z = 2)
  - Sum parameter DSTYSLSSTLTLSK (*m/z* 751.8828; z = 2)

Method validation

Table 1: The LOD and LOQ are defined as S/N = 3 and S/N = 10. Reproducibility was determined on the same day and repeatability on three different days. Samples were analyzed as triplicates. The linearity was determined from the LOQ to 15 and 45 mg/sample, respectively. The test of linearity was performed according to Mandel with a statistical safety of 99.9%.

Peptides for	LOD (µg/sample)	LOQ (µg/sample)	Reproducibility (%)	Repeatability (%)	Linearity (mg/sample)
Rituximab	9.6	33	88 ± 7	92 ± 6	15
Trastuzumab	4.4	15	105 ± 1	97 ± 7	15
Daratumumab	1.4	4.7	106 ± 5	109 ± 4	15
Sum parameter	25	79	97 ± 0.8	95 ± 4	45

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