**Introduction**

- Mass-based fraction collection is a highly selective technique for compound purification. In contrast to fraction triggering with a less specific detector such as an UV detector, fraction collection can be triggered on a specific target mass with a mass selective detector (MSD). Consequently only one fraction containing the target compound is collected in each run and no redundant fractions need to be sorted out which saves time and resources.

- Herein we demonstrate that mass-based fraction collection can be excellently employed for the purification of crude synthetic peptides. Our 12 different test samples were routinely synthesized at Cancer Research UK for therapeutic purposes. They:
  - range in size from 1 to 10 kDa
  - have isoelectric points between 4 and 13
  - cover hydrophilic and hydrophobic peptides

- Depending on the sample amounts peptides were purified by reverse phase HPLC either on analytical scale (1 mL/min, column with 4.6 mm i.d.) or preparative scale (up to 15 mL/min with columns up to 21.2 mm i.d.).

- Water and acetonitrile were chosen as eluents, both containing 0.08% TFA as ion-pairing agent.

- In order to save time in routine purification a gradient was chosen that elutes the sample/fraction volume, flow rate and delay volume.

**Results and Discussion**

- Fraction collection is triggered on the DAD-signal (threshold and slope) and a target mass.

- Start and stop of fraction collection are indicated in the chromatograms by green and red lines, respectively. Collected fractions are labeled with the corresponding vial positions in the fraction collector and all found masses.

- Chromatograms of the re-analyzed fractions demonstrate that the fractions were reliably collected. ESI-mass spectra can be easily extracted from TIC chromatograms. They can be used to further proving the identities and purities of the isolated peptides.

**Instrumental Set-up**

- In order to achieve best performance with the Agilent 1100 Series modules for analytical (< 5 mL/min) as well as preparative scale (> 5 mL/min) purifications dedicated systems can be assembled. These systems are optimized regarding: sample/fraction volume, flow rate and delay volume.

- For robust mass detection the Agilent 1100 Series LC/MSD SL quadrupole mass spectrometer system was employed.

- The Agilent 1100 Series Purification System is controlled by the well established Agilent ChemStation Software. The Purification/HighThruput Software controls the purification process and keeps track of all collected fractions.

- Reliable fraction collection is ensured by a fully automated delay volume calibration that accurately calculates the time delay between peak detection and fraction collection (integral part of the platform).

- The instrumental set-up comprises two flow paths. The main flow leads from the pump(s) to the autosampler, the column, the diode array detector (DAD) and the Active Splitter before reaching the fraction collector. Since the MSD is a destructive detector and the flow rate of the main flow is too high to route it directly into the electrospray source, a make-up flow is sustained by an isocratic pump. This make-up flow leads from the isocratic pump to the Active Splitter before reaching the MSD.

**Conclusions**

- Mass-based fraction collection is a highly efficient technique for high-throughput purification of crude synthetic peptides.

- Due to an accurate delay volume calibration fraction collection with the Agilent 1100 Series Purification System is highly reliable.

- The presented system can cope with all kinds of peptides ranging from small to large, from hydrophilic to hydrophobic, from acidic to basic.

- Fraction collection triggered on predefined masses is advantageous over conventional triggering on less specific detectors signals since only the compounds of interest are collected in each run. No additional time needs to be spent to pick out target compounds out of a series of redundant fractions. Furthermore no fraction collector resources are wasted.

- No additional preparation steps are needed to obtain mass spectra within chromatographic runs. Peptide characterization with an ESI-MSD therefore is an online alternative to MALDI-TOF.