OVERVIEW

A generic assay to analyze low-abundance HCPs for a wide range of recombinant biopharmaceuticals is presented [1]. The HCP assay includes two types:

1. Discovery Assay
2. Monitoring Assay

The assay overcome limitations associated with current analytical methods for measuring HCPs (e.g., ELISA, gels, blots), and offers a complementary approach to provide quick and comprehensive information on the identity and quantity of each individual HCP in the sample.

The analysis results for different therapeutic protein samples are included, and the comparison against ELISA analysis is provided.

2D-LC METHODS

System: nanoACQUITY™ UPLC® system with 2D technology.
A reversed-phase/reversed-phase (RP/RP) method was developed that uses the pH of the mobile phases to change the selectivity of a peptide separation in two separate dimensions [2,3].

1st dimension: Separation performed at pH 10
   - Column: 1.0 mm x 50 mm BEH C18, 1.7 µm
   - Flow rate: 12 µL/min
   - Mobile phases:
     - A: 20 mM ammonium formate in water
     - B: ACN
   - 10-step fractionation: 1. 10.0%, 2. 12.4%, 3. 14.0%, 4. 16.4%, 5. 18.0%, 6. 20.6%, 7. 23.2%, 8. 25.4%, 9. 27.6%, 10. 30.0%

Online dilution: 10-100 dilution (0.1% TFA) of the eluent from 1D before analyte trapping onto the trap column.

Figure 1. Fluidic configuration for 2D chromatography with on-line dilution: (A) Sample loading; (B) Peptide elution from 1D; (C) 2D trapping.

1. Catalin, et. al., Waters Corporation, Milford, MA 01757, USA; 2R&D and Pilot Services, Novasep Process Development, Metz, France

REFERENCES

CONCLUSIONS

The discovery assay allows the identification and quantification of low-abundance HCP contaminants in biopharmaceuticals over five orders of magnitude in concentration.

UPLC in combination with the TO-S tandem quadrupole shows great promise for high-throughput HCP quantification on microliter scale samples.

Protein A purification of mAbs using different purification protocols produces different HCP patterns. The measured HCP concentrations in current study show great agreement with ELISA assay.