Table 15: Successful On-line Interfacing of MS with Traditionally Non-MS Compatible LC Methods

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Scope:
The number of novel bio-therapeutic antibody-based formats in drug development is continuously increasing. Characterization of these formats is challenging. Since established physiochemical and mass spectrometric methods show limited capabilities for characterization of product related impurities new analytical strategies must be developed. Discuss the needs and benefits for the combination of classical biochemical separation methods with high resolution mass spectrometry.

Topics for Discussion:
1. Do you already use methods like SEC-MS, IEC-MS or even CE-SDS-MS in your organization and if yes? Are you currently developing or do you already use it routinely in your lab?
2. What are the main pain points developing it?
3. What is the main application e.g. characterization, trouble shooting, CQA assessment?
4. Do think it could be also used for release purposes like MAM?
5. What other combinations could you imagine (e.g. Boronate, HIC etc.)?
6. Are you also interested in structure/function MS feasible methods (e.g. FcRn, FcGammaRIIIa etc.)?
7. How is the experience with combination of different vendors?
8. What are the advantages or disadvantages compared to 2D LC-MS couplings?
9. Do you think this combination can replace the traditional methods?

Discussion Notes:
1. Do you already use methods like SEC-MS, IEC-MS or even CE-SDS-MS in your organization and if yes (1a)?
- Roche: most important combination is SEC-MS
- Seattle Genetics, Novo Nordisk: CE-MS experience, Zip Chip, only small molecules by now
- Waters: not pushing CE, not a focus area
- All: interest in affinity CE MS

1a) Are you currently developing or do you already use it routinely in your lab?
- Seattle Genetics and Novo Nordisk: bought Zip Chip
- but probably not used as routine method, more research, to answer specific question
- Seattle Genetics: HIC separation with ADCs,
- for release of ADCs: SEC MS on routine basis, as a desalting method

2. What are the main pain points developing it?

3. What is the main application e.g. characterization, trouble shooting, CQA assessment?
- intact mass, not denatured, native spectrum is clearer, less charged species
- may be used as release in the future
- no sample prep: confidence not to induce deamidation?
- Intact will be needed since it's in some cases the only method to see certain fragments, modifications, for instance modifications of linker from ADCs

4. Do think it could be also used for release purposes like MAM?

5. What other combinations could you imagine (e.g. Boronate, HIC etc.)?
- boronate/affinity for fractionation of glycation species

6. Are you also interested in structure/function MS feasible methods (e.g. FcRn, FcGammaRIIIa etc.)?
    - Roche: experience with FcRn column online MS, for homo/heterodimer of Met250
    - Roche: experience with target antigen column

7. How is the experience with combination of different vendors?
    - a lot of experience, combining different modules: Autosampler, UPLC, MS
    - works most of the time, but better a dedicated instrument for one analysis
    - 4-5 people for 6 instruments

8. What are the advantages or disadvantages compared to 2D LC-MS couplings?
    - Charge: probably not a good solution for all mAbs, depending on the pI
    - SEC straight forward

9. Do you think this combination can replace the traditional methods?
    - unclear, needs time and invest to establish
    - probably not a hardware restriction, but buffer system
    - needs intense support at the beginning
    - needs an experienced service technician from the vendor

    - Future topic: real time release