Table 3: Development of Chromatographic Methods; The New and the Old

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Scope:
Continuous innovation in chromatography, method development and sample preparation help analytical laboratories to use their resources better. Working with highly complex samples or trying to increase sample throughput requires the implementation of recent advancements. Benefits and limitations of automated sample preparation and the utility of novel reagents (e.g. derivatization, labelling, digestion, etc.) will be discussed. Besides developing a proper sample preparation, the optimization of the separation may require significant efforts. Traditional approaches will be compared to computer-assisted method optimization. Instrumentation has an important role in reducing analysis time and increasing resolution, significantly contributing to the success of the overall method development. The variety of instruments´ designs available provide more possibilities but also may complicate method transfer. Finally, with latest biopharma products chromatographers face unprecedented challenges. The applicability of generic methods and developability of separation methods for new biologics will be discussed.

Questions for Discussion:
1. Which sample preparation methods do you use in your laboratory and how do you increase sample throughput?
2. How do you develop a separation method?
3. How do instruments designs help/complicate your method development/transfer?
4. How do you address method development issues when analysing new biologics?
5. Do pharmacopoeia methods play a role - at all - in method development?

Discussion Notes:

Sample Preparation Methods

- Analysis of excipients is important
- Sample clean-up can be applied if surfactants disturb separation
    - In particular for (high concentration) antibody solutions
- Sample preparation is mainly based on trial & error approach
- Detergents are frequently problematic
    - Stick to columns
- No standard recipe yet to get rid of detergents
- Immobilized enzymes
    - Robust, recommended
    - Used for screening purposes (e.g. formulation experiments)
- Buffer exchange can change sample consistency (e.g. reversible aggregation)

Use of Guard Columns

- Not necessary, if the DP is pure
- Necessary, if the column must be protected
- Care should be taken, when using for SEC
    - Sample consistency may change on the pre-column

How to proceed in method development

- Trial & error approach is still frequently used, especially resources are limited for method development
- Application of full factorial designs is often time consuming
- OFAT approach is generally applied, if there is enough time
- Use of method development software (e.g. Chromsword)
    - Good documentation by the automated system, implementation of artificial intelligence
    - Used for screening purposes (e.g. IEX and gradient-based methods)

How do you screen columns?

- No particular approach, own favorite columns preferred
- Columns from well known, established providers preferred (no "exotic" columns are chosen)
- Testing should include different batches (!)
    - To avoid development at the edge of columns´ performance
- Column interchangeability:
    - More than one candidate is tested during method development (column screening)
    - For biologics generally not taken into account

Samples for method development

- Use of artificially stressed samples
- Use of samples stressed by freeze/thaw cycles

Bio inert Systems

- HPLC is still frequently used
    - up to 25 years
    - as long as it works
    - as long as it is OK
- HPLC/UPLC are both used in method development
    - Comparison of performance
    - Why change an **existing** system without a reason to change?
        - Robustness may decreas with increasing performance
        - New peaks may appear
        - QC uses HPLC
        - Switching from HPLC to UPLC for a **new product / new method**
            - (+) saves solvent and time

If a new Biologic enters the Lab:

- Robotic systems can be used for screening purposes
- Platform knowledge is used (standard methods are applied, if possible)
- Non-platform biologics are in most of the cased challenging
- Many methods are needed for all quality attributes to be addressed
- Strategy used depends on size of the Company

SOPs / Protocols in Method Development?

- Method development cannot be standardized
- In research: standard parameters are tested (but workflow not standardized)
- Standard screening solutions for gas chromatography only
- LC screening linked to projects

Time for Research?

- Company dependent
- Big pharma: yes, even publications
- Small companies: no capacities for real R&D

Are Development Kits useful?

- Not interesting

Are Pharmacopoeial Methods useful in Method Development?

- In some cases yes (e.g. for assay)

How are SST Criteria being set?

- Only **after** validation meaningful criteria can be set
- Only if historical data exist
- As knowledge on the molecule is gained more criteria can be defined
- SST criteria
    - Resolution; most critical aspect
    - Similarity; related to reference standard
        - Visual evaluation
    - Tailing factor
    - Area is usually not considered in A% methods
- The same reference standard should be used throughout development
- Information should be shared between departments

TAKE HOME MESSAGES

- Share opinions with colleagues, the knowledge of others is very important
- Method development can be automated to a certain level, but workflows cannot be standardized and depend on the specific situation / behavior of the molecule
- Robustness of the method is more important than best available resolution of the species