Probing Deep Understanding of an LC-UV-MS Method for Characterising Oligonucleotides

Challenge

Method development to deliver a simplified platform method for assay and impurities for oligonucleotides (an important emerging class of gene targeted therapeutics) is not straightforward.

The complexity of oligonucleotide structures and the large numbers of non-resolvable impurities and stereoisomers involved require both UV and MS dimensions for quantification in Liquid Chromatography (LC). The method involves multi-step manual data processing and subjective interpretation by the analyst at some key stages. Thus detailed training is required to ensure analysts are competent, both in-house and at partner organisations.

In this case, the accelerated timelines for lead oligo drug product New Drug Application (NDA) filing did not allow for full development of a simplified method, so deep understanding of the existing method was required to drive method improvement and standardisation.

Approach

A facilitated Britest study was conducted, to identify opportunities to remove subjectivity from the method, which was believed to be leading to deviations, inconsistent results, and System Suitability Test (SST) failures.

Breaking the analytical method down into a flowchart, a tailored form of Britest Duty Definition and Equipment Specification (DuDES) analysis was used to develop a risk prioritisation and mitigation strategy for each task needed to analyse the drug product. By identifying what each task *must have or attain* and conversely *what must be avoided*, the project team were able to generate and discuss a set of potential actions to mitigate the risks identified.

Benefits

- Harmonisation across departments and with external partners.
- Identification of actions to improve training materials and internal standardised method.
- A standardised method is beneficial for in-country testing, i.e., where drug product is tested by government labs without a formal test transfer.

Key Features:

Client - Multinational pharmaceutical manufacturer

Industry - Application Area

Pharmaceuticals - Analytical method improvement and standardisation, Operational excellence

Challenge

A simplified platform method for determining assay and impurities for oligonucleotides is desirable but the accelerated timelines for lead oligo drug product NDA filing did not allow for full development of a simplified method.

Solution

Deep understanding of the existing method can be used to inform improved training and method standardisation. The Britest methodology was used to complement established methods of Analytical Risk Assessment (ARA) in this respect.

Outcomes

A facilitated Britest study based on a tailored DuDES analysis identified opportunities to remove subjectivity from the method, leading to harmonisation across departments and external partners, identification of actions to improve training materials and the internal standardised method, and consequential benefits for in-country testing.

Task	Prepare mobile phase glassware		Task purpose	Reduce residual metal (mainly sodium, potassium, iron) ions - reduce adduct formation in MS spectra; required to pass the Na adduct SST criterion			
Category	Must have	Desirable	Avoid	Prefer not	Identified risks	Priority (1= Low, 4 -= Critical)	Mitigation steps
Humans ("Manpower")	Accurate following of procedure. Detailed process is well described in procedure	Planning - 12 hours required for procedure	Stock outs of glassware Residual metal ions present		Sodium ions - failure not seen acc. to team	2	Dependent on quality of glass washing procedures.
Machinery / equipment	Amber glassware (amber is an external coating)		Amber glassware being replaced with clear glass due to availability	Inconsistent training is consistent (in-lab practical divergence)	Differences in practice between departments	1	**Action 4: Understand why amber glassware was originally specified in the method. Document in method development history report if necessary.**
Methods	Appropriate level of detail in procedure	Filling levels, stirring requirements		Different parts of the instructions split across different documents	Operator to operator variation in practice	2	**Action 5: Bring everything into one in-house standard method**

Extract from DuDES analysis focusing on mobile phase glassware preparation

> ***DuDES is a promising technical deep dive tool to complement our existing risk assessments.***
>
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Generating value from process understanding

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