Mass Spectrometric Analysis of Process Related Impurities

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Introduction

The production process of any biological drug requires the use of a wide range of chemicals such as nutrient media, initiators of protein production, selection processes (e.g. antibiotics) as well as chemicals used in the subsequent purification processes. Furthermore, any chemical modifications to the naturally produced biologic, such as PEGylation, will result in an even larger number of chemicals coming into contact with the drug product. All of these extraneous chemicals can end up in small amounts in the final product (despite the significant purification strategies applied during downstream processing) and may be capable of generating a biological response such as an immune reaction or other adverse event in a patient and therefore putting them at risk. Furthermore, the product itself may be at risk from extended exposure to these chemicals, such as increased levels of oxidation or aggregation or other chemical modification. Changes to the structure of the product as a result of the action of these residual levels of chemicals can result in a decreased shelf life, increase in aggregation or other adverse effect, resulting in a loss of efficacy.

The need for control and monitoring of the levels of these so-called process related impurities is not a new consideration. It was clearly described in the ICH Q6B guidelines for production of biological molecules, a document that was taken up by both the EMA and FDA 20 years ago [1]. This document divides process related impurities into three categories: cell substrate derived impurities (protein/nucleic acid derived from the host organism), cell culture derived impurities (such as inducers, antibiotics and other media components) and down-stream derived impurities such as enzymes, chemical and biochemical processing reagents (e.g. cyanogen bromide, reducing agents), inorganic salts, solvents, carriers, ligands and other leachables.

To this end, the concentrations of chemicals involved in the bioproduction process must be controlled. Monitoring using specific assays must be performed to make sure that the purification processes employed remove these chemicals and they are demonstrably at or below the maximum set or permitted levels and in line with any product specific regulatory guidelines.

Different process related impurities will require different types of assay but one technique which has numerous applications in the area of process related impurity analysis is mass spectrometry. This technique allows the detection, by means of mass, of the chemical of interest, either in its native state or a chemical derivative thereof, which can be produced as part of the experimental workup for detection. Mass spectrometry is a highly sensitive and selective technique which is capable of detection down to ppb levels, depending on the matrix in which the chemical of interest is present.

The primary requirement for any form of mass spectrometric analysis is the ability to ionise the molecule under investigation. Many methods of ionisation are available and these need to be matched with the inlet method (usually either Gas Chromatography (GC) for volatile and semi-volatile components or Liquid Chromatography for almost everything else) and an appropriate type of mass separation and detection system. Using the wide range of hyphenated mass spectrometry instruments available allows the quantitation of a wide variety of process related chemical impurities.

In order for ionisation to take place in the source of the mass spectrometer, chemical work up processes may need to be employed to derivatise the molecule of interest. The aim is to produce a volatile form of the residual chemical which can readily enter the gas phase within the source of the mass spectrometer allowing ionisation to take place with subsequent passage through the mass analyser to the detector.

The intensity with which a signal is detected at the expected mass can be used as a means of quantitation. This may be achieved through either spiking experiments to determine the

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degree of response for known amount of the chemical of interest in the matrix or through the demonstration of linearity of response by analysis of different amounts of the chemical of interest.

It is important to bear in mind that different buffer systems and matrices (such as would be expected to exist at different points of the purification process) can have an impact on the ability to detect the residual chemical due to the presence of many other components which could affect ionisation or any derivatisation step that may be required. Therefore, each sample matrix (i.e. stage of the purification process) must be individually assessed to determine the extent to which the compound of interest can be detected.

The examples given below demonstrate the applicability of mass spectrometric analysis across a range of commonly occurring classes of chemicals which require monitoring. It should be stressed that monitoring levels of at least the key chemicals of interest is something that should be performed as the purification process is being developed, helping to ensure that the processing is optimal and appropriate as a means of not only purifying/concentrating the product of interest but of removing the process related impurities.

Inducers - IPTG

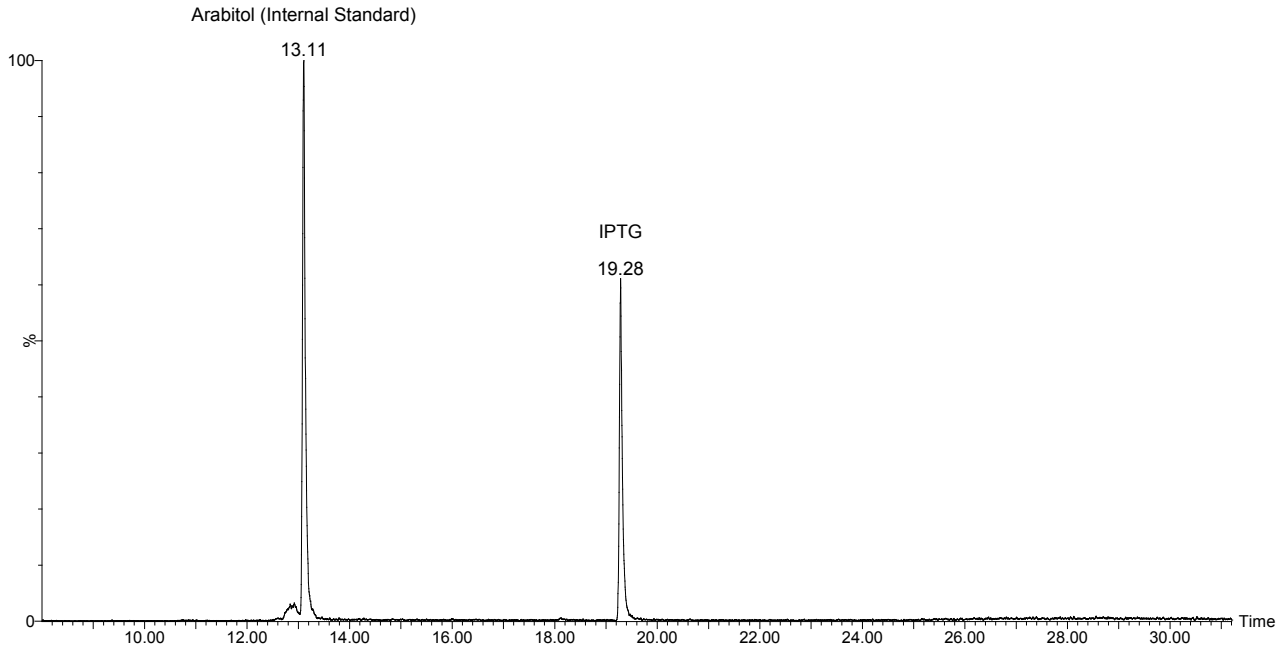
IPTG (isopropyl–β-D-thiogalactopyranoside) is used as an inducer of protein expression, triggering transcription through the lac operon. Chemically, it is a derivative of the monosaccharide Galactose and acts as molecular mimic of the disaccharide Lactose. Since it is effectively a modified monosaccharide, a monosaccharide analysis type approach works well for quantitation.

The process utilised by BioPharmaSpec involves derivatisation and analysis by gas chromatography-mass spectrometry (GC-MS). The derivatisation process produces a volatile derivative of the otherwise non-volatile IPTG, which is then ionised and fragmented by electron impact mass spectrometry in the source of the instrument. Knowing the structure of the IPTG derivative and the fragmentation pathways that are followed during ionisation, it is possible to identify key fragment ions and screen the data for these signals. Prior to entry into the source of the mass spectrometer, the sample is separated into its constituent components by gas chromatography. Thus, fragment ions must be identified at the retention time expected for derivatised IPTG in order to be positively identified. The use of standards allows identification of retention time and fragmentation pattern.

Cell selection mechanisms - Kanamycin

Kanamycin is one of a number of antibiotic drugs used for cell selection purposes, ensuring that only the cells that have been successfully transfected will grow. Residual Kanamycin can be analysed following chemical derivatisation of the four amine functional groups. The size and structure of the derivatised molecule lends itself to analysis using on-line liquid chromatography-mass spectrometry (on-line LC/ES-MS) with electrospray ionisation. Furthermore, fragmentation of the intact molecule produces a number of strong fragment ions. This fragmentation pathway can be used as a way of monitoring Kanamycin, using a triple quadrupole mass spectrometer. This form of mass spectrometer is capable of allowing only ions of certain mass to pass through the quadrupoles (the mass separation

Figure 1: GC-MS analysis of derivatised IPTG. Arabitol is used as an internal standard for purposes of quantitation.



regions). As such, ions can be filtered according to the mass of the intact component and then the fragment ion that is produced. This process of mass selection through production and selection of a fragment ion is known as reaction monitoring. The same procedure can be applied to the use of an internal standard (such as another antibiotic not used in the manufacturing process) which undergoes its own unique fragmentation process in a similar way. The molecular transition of the internal standard can be monitored in the same way and at the same time as the Kanamycin. This is known as multiple reaction monitoring (MRM). Since the molecule of interest is effectively filtered twice, background ions are very effectively eliminated, resulting in very high sensitivity (ppb).

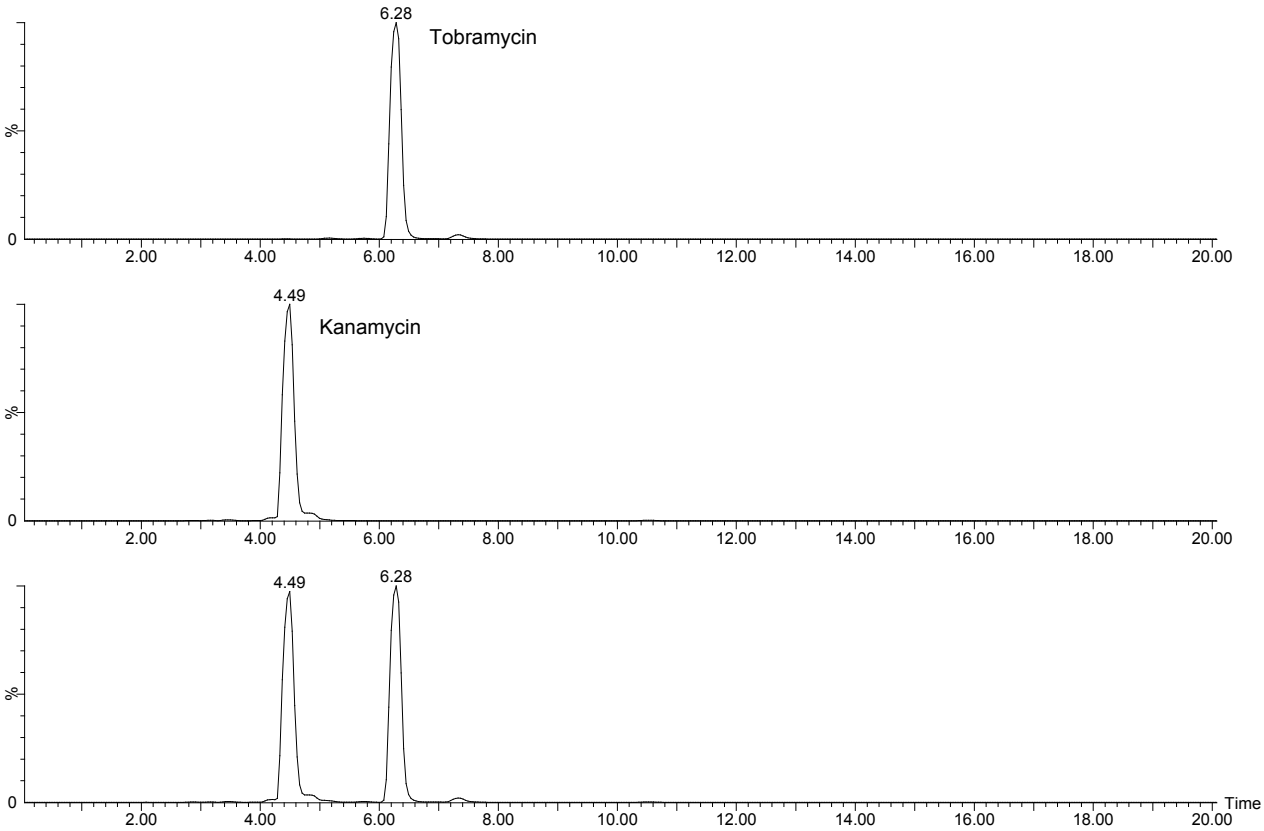
Polymeric species - Polypropylene glycol (PPG)

The process of drug production and formulation often involves the use of polymers, such as polypropylene glycol (PPG), as antifoams. The nature of these molecules is somewhat different from other types of chemical with a specific, defined structure. In these cases the compound of interest is composed of a range of similar structures centred around a particular mass, but varying in the number of repeating units in the chain. Molecules of this type are also perfectly amenable to mass spectrometric analysis. Structurally, this class of molecule is a polymer of ether linked units with side chain methyl groups and capped with hydroxyl functional groups. The length of the polymer can vary, giving rise to different size populations of commercially available compounds. These molecules are amenable to analysis using on-line liquid chromatography-mass spectrometry (on-line LC/ES-MS) with electrospray ionisation (in a similar way to Kanamycin) or atmospheric pressure chemical ionisation. However, the nature of their chemical structures means that no derivatisation is required to optimise ionisation or assist in the generation of readily identifiable fragment ions. Since in this case a population of molecules is being investigated, it is useful to monitor molecular transitions in the same way as for Kanamycin. However, the different polymeric species will separate as a result of the chromatography. These separated components will enter the mass spectrometer over a period of time and each will have a unique transition which can be monitored. Triple quadrupole mass spectrometers are therefore used for analysis of this class of molecule since MRM parameters can be set up to constantly monitor the expected transitions of the various species (as determine in the initial development of the method) through the course of the chromatography.

Mass spectrometry based methods can also be used to assess biopharmaceutical products for other process derived residuals including:

- Stabilisers and antioxidants such

Figure 2: On-line LC/ES-MS analysis of derivatised Kanamycin. Tobramycin is used as an internal standard for purposes of quantitation. The upper ion chromatogram shows the molecular ion transition monitored for Tobramycin, the middle ion chromatogram shows the molecular transition monitored for Kanamycin. The lower ion chromatogram shows the sum total of ion transitions monitored.



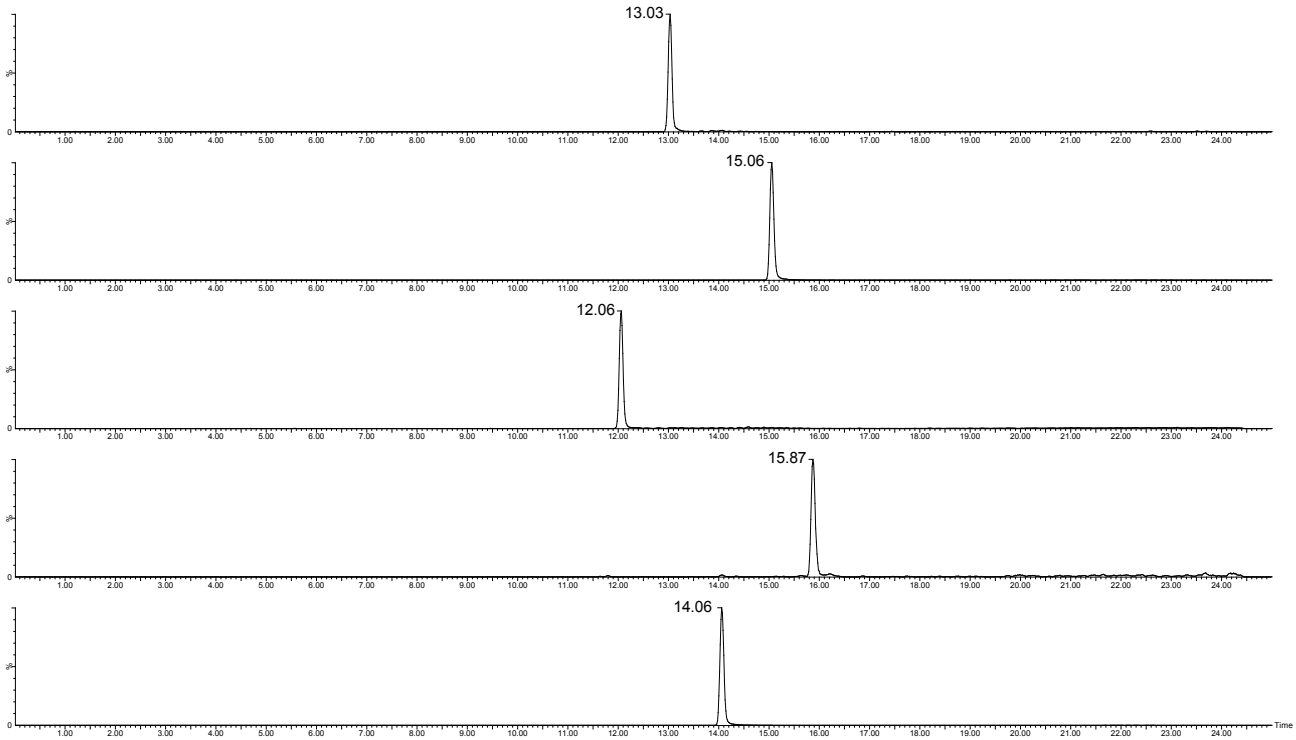


Figure 3: On-line LC/ES-MS analysis of PPG oligomers. Each chromatogram shows a specific ion transition.

as Ascorbic acid and sugars.

- Solubilisers such as Amino acids, Surfactants (Tween, Polysorbate, Triton) and Denaturants (Guanidine, Urea)
- Reducing agents such as Glutathione, DTT etc, and
- Chelating agents such as EDTA

As well as the direct monitoring of residual levels of chemicals used during the manufacturing process, this type of high sensitivity analysis can be used, if necessary, to monitor residual levels of chemicals present within the raw materials themselves.

Conclusion

Monitoring of residuals is of key importance in the control of process related impurities and is a requirement of the ICH Q6B guidelines, a document adopted by regulatory agencies. If product quality is to be assured, it must be demonstrated that residuals are within agreed and well-defined limits. Furthermore, assessment of the levels of residuals at different stages of purification is important to help define the process of product manufacture. The techniques used for monitoring of a particular component must be sufficiently specific and sensitive such that low levels of the chemical can be readily detected and changes in those levels easily observed. Mass spectrometry, when combined with suitable chemical procedures and chromatographic separation, proves to be a highly sensitive and versatile tool for the detection of low levels of residuals due to its ability to separate and precisely select for masses of interest, even when handling complex mixtures.

References

1. ICH Guideline Q6B: *Test Procedures and Acceptance Criteria for Biotechnological/ Biological Products.* International Council on Harmonzation of Tehnical Requirements for Pharmaceuticals for Human Use, Geneva, Switzerland, 1999

Dr. Richard Easton is Technical Director at BioPharmaSpec Ltd

Richard obtained his PhD in glycoprotein structural characterisation using mass spectrometry from Imperial College of Science, Technology and Medicine. He subsequently spent several years there as a postdoctoral research scientist working in the field of glycoprotein structural characterisation with emphasis on glycan elucidation. He moved to GlaxoSmithKline for a short time where he was head of mass spectrometry for the toxicoproteomics and safety assessment group. Richard joined M-Scan Limited (now part of SGS Life Sciences) as a biochemist and became the Team Leader for Carbohydrate Analysis before being appointed Principal Scientist. Richard joined BioPharmaSpec in 2016 as Technical Director for Structural Analysis and is responsible for management of all aspects of carbohydrate and glycoprotein characterisation at the primary structure level.

Dr. Andrew Reason is CEO and MD at BioPharmaSpec Ltd

Andrew is the founder, CEO and MD of BioPharmaSpec. He has 25 years of experience in analysis of both novel and biosimilar biopharmaceuticals and has been involved in the commercialisation of a number of analytical methods for characterising proteins. In addition to his scientific and managerial duties, Andrew has contributed to many industry publications and is a regular presenter at conferences. Andrew is also currently a Visiting Professor at the University of Warwick.