

Trace analysis and the challenges for high sensitivity analytical methods

A recent symposium looked at the use of highly sensitive methods in pharmaceutical analysis. **Joseph Chamberlain** reports

Given that many drug substances and impurities are highly potent and can exhibit both desirable and undesirable pharmacological or immunological effects at very low doses, trace analysis is important in the licensing of safe medicines, said Malcolm Dash, of the Medicines and Healthcare products Regulatory Agency, in an introductory talk on what regulatory authorities would be looking for in this area of analytical application. In particular, new requirements were emerging in the areas of genotoxicity, heavy metals and mycotoxins.

Increasing emphasis is placed on the control of potential genotoxic impurities that may be present at low levels in a drug substance and which can cause damage to DNA that may be associated with mutagenic or carcinogenic activity. The challenge facing industry and regulators is to determine the safe level of such substances. The regulatory dossier must include discussion of potential genotoxic or carcinogenic impurities arising from synthesis of the drug substance, said Mr



Malcolm Dash: researchers should challenge current knowledge

Dash. Specific guidance has been developed on the control of residual metals from catalysts and reagents used in the synthetic route. Heavy metals can also be taken up from the soil, water or air by plants used in herbal medicines. The European Pharmacopoeia includes details of a non-specific test method for heavy metals with sensitivity down to 1–2ppm, although the test is not applicable to

all metals. Mycotoxins are secondary metabolites of moulds produced by fungus and include highly toxic, carcinogenic, mutagenic and teratogenic compounds. Proliferation of fungal growth due to use of poor drying processes, ineffective fumigation or poor storage of harvested crops can lead to high levels of mycotoxins.

Other areas of concern for the regulatory authorities included agricultural pesticides subject to stresses during storage and transport, residual proteins in biotechnology products, and extractables and leachables in packaging. Cleaning validation is an important consideration in the manufacturing environment. Potential for cross contamination with products and residues of cleaning agents and disinfectants needs to be minimised, particularly where there is potential for hypersensitivity reactions.

Researchers should challenge current knowledge, consider the implications of any analysis so that the ultimate aim of patient safety is achieved, concluded Mr Dash.

Approaches from the pharmaceutical industry

Speakers from the pharmaceutical industry discussed the technology available for high-sensitivity measurements. Trace analysis requires sophisticated analytical technology to obtain the desired sensitivity. At the level of 100ppm, liquid chromatography with UV detection, nuclear magnetic resonance and gas chromatography with flame ionisation detection are often adequate but for the analysis of genotoxic impurities GC-MS or LC-MS is required, said Andrew Ray, of AstraZeneca, Loughborough. Different ionisation techniques now include the well established electrospray for peptides and proteins, as well as atmospheric pressure chemical or photo ionisation for less polar molecules. A feature of modern mass spectrometry as a qualitative tool is the potential for accurate mass measurement of single ions. For example the ions derived from $C_{14}H_{16}N_4OSF_2$ ($m/z=327.1091$) and $C_{12}H_8N_4OS_2F_2$ ($m/z=327.0186$) could be readily distinguished. Most quantitative mass spectrometry work is performed by single ion monitoring on a quadrupole analyser. The equipment is sensi-

tive, specific, robust and relatively cheap. As it is set to allow ions of a single mass through, it acts as a mass filter and is not a scanning instrument, so that a faster duty cycle is obtained. It is easily interfaced to GC or HPLC and has a reasonable linear range. New ionisation techniques resulting in direct analysis in real time are being produced which can analyse solid samples. For formulation development this may mean that no sample pretreatment is required. There are still issues around sample suppression and complexity of spectra produced, but rapid quantitation may be possible, concluded Mr Ray.

Quantifying trace impurities

Karen Taylor-Worth, of Pfizer, Sandwich, Kent, reviewed analytical strategies for the quantification of trace impurities, highlighting approaches developed at Pfizer, including a generic method for determination of a range of potential alkylating agents. Pfizer adopts a staged risk management and control strategy based on the principles of the threshold of toxicological concern, modified where necessary for therapeutic class and patient class. The strategy involves excluding the matrix from the chromatographic system, maximising the transfer of analyte into the chromatographic system, maximising the vol-

ume handling capabilities, and increasing the use of automation. Alkylating agents are highly reactive and have a broad range of physicochemical properties so specific methodology for each species is usually required. A generic analytical procedure applicable to a range of alkylating agents was developed, employing an *in situ* derivatisation step followed by headspace GC-MS.

Pfizer is also involved in a collaborative venture with the Product Quality Research Institute (PQRI), a process involving the US Food and Drug Administration's Centre for Drug Evaluation and Research (CDER), the pharmaceutical industry and academia. The mission of the PQRI is to conduct research to generate specific scientific information that should be submitted in a regulatory filing to CDER. There has been growing concern expressed by regulatory authorities with respect to the potential generation of genotoxic impurities as a result of interactions between sulphonic acids and alcohols. Some studies have been carried out in house within the industry showing some understanding of the levels of sulphonic esters formed under synthetically relevant conditions. A clear challenge was for industry to build on these studies and develop a fundamental understanding, said Dr Taylor-Worth.

This symposium organised by **The Joint Pharmaceutical Analysis Group** took place at the School of Pharmacy, University of London, on 13 December 2007

Ways to carry out trace analysis in practice

In analytical chemistry, a trace element is an element in a sample that has an average concentration of less than 100 parts per million atoms, or less than 100 µg/g (100 mg/kg), said Andy Pickett, of Ipsen Biopharm Ltd, Wrexham. The use of biological macromolecules — proteins — for therapeutic purposes is now part of routine treatment for many medical conditions. Many of these proteins have activities at trace levels, parts per million or lower, and therefore their analysis presents challenges. Nevertheless, advanced technology has been used for the routine analysis of these products. Analysis of molecules with such potency has followed routine practices for proteins, but the materials must be handled under special containment conditions, especially when high concentrations are needed to obtain an analytical response. More recently, powerful analytical tools, such as mass spectroscopy and surface plasmon resonance, have been applied to their analysis, both as pure neurotoxins and as protein complexes. The therapeutic toxins currently available are usually complexed with numerous other proteins as natural products, meaning that the use of such technology can be viewed as a real advance. Biological activity can be demonstrated in a range of models at parts per trillion concentrations or lower in order to provide assurance of the quality, efficacy and safety of these products for routine therapeutic use.

For potency assessment of botulinum toxin in a drug product, the classical measurement by an LD50 test in mice is controversial. Alternative assessment by enzymatic determination has sensitivity issues. A recently developed method for the detection of

neurotoxin activity uses mass spectrometry of the products of a cleaved substrate.

Analysis of exceptionally low concentrations of biological actives as drug product presents significant difficulties so that new technologies or *in vivo/in vitro* systems must be employed, concluded Dr Pickett.

Over the past few years, genotoxic (and potentially genotoxic) impurities (PGIs) have become a significant issue within the pharmaceutical industry, said Andrew Baker (AstraZeneca, Loughborough). Typical compounds that may be genotoxic are alkylating agents, epoxides and nitrosoamines. As a component part of AstraZeneca's strategy for managing the issue of PGIs, an external collaborative exercise has been established with a leading trace analysis group. The aim of this collaboration was to generate an understanding of the applicability of different analytical techniques across the entire range of analytes found in PGI analysis. With this information the most suitable technique can be readily identified and applied to any PGI analysis, negating the need to develop methods from scratch. Significant resource savings could be realised using this approach and the generation of robust analytical data could be achieved within the challenging timeframes set by the R&D organisation, concluded Mr Baker.

When working with highly sensitive methods, it is inevitable that demands are made at the limit of detection of the technique. How to report values in this twilight zone is an ongoing debate for analysts and the users of the data generated. Steve Ellison, LGC, Teddington, discussed such statistical challenges in trace analysis. The purpose of detection limits is to show a lower operating

limit and to set rigorous decision and control limits. It is important, he said, to understand the basic concepts in the statistical treatment of data. Thus a critical value is the instrument response used to trigger action and is an observation; the detection limit, the amount of substance leading to action, is the truth; and the quantitation limit, the lowest level at which uncertainty is acceptable, is arbitrary.

It must be recognised that even true zero values come with standard errors and therefore to regard negative readings as equal to zero, or to ignore them, will result in the true zeros being reported as a positive average. Thus although negative values would appear to contradict common sense, they must be reported. He further contended that obtaining a value less than the limit of detection does not imply an invalid result and one should report the raw result and its uncertainty if possible. A number can be processed; a qualitative statement can not. Not all systems provide readings below thresholds and there is a case for a different approach, such as the determination of the maximum likelihood estimation, a statistical procedure not easily useable in the pre-computer age, but now readily available in software packages.

Detection limits are based on statistical reasoning. When determined during validation they are indicative for typical in-house validations, and approximate values are usually adequate. Decision limits on which action depends should be rigorously checked and monitored regularly. Report raw values if you can, investigate censored data methods if you can't, and remember that a detection limit is something to stay well away from, concluded Dr Ellison.

Manufacturing and packaging as sources of impurities

When deciding on an approach to cleaning verification and to setting specifications, several aspects need to be considered said Tim Wood, of GlaxoSmithKline, Harlow, Essex. These include the type of plant, contact areas, properties of the active ingredient and the next use of the plant. Potency of the active ingredient is an issue, as is its stability and its nature, such as its allergenic properties. Factors to be considered regarding other use of the plant include batch size and drug potency.

Specifications can be set on a generic, worst case scenario or by taking some (or all) instances individually and generating specific specifications. Generic specifications are easier and quicker to apply, there is less chance of making an error and they tend to err on the side of caution. However they may result in unnecessarily tight specifications and very high potency ingredients may still need to be considered individually.

Tailored specifications offer a better guarantee that appropriate specifications are set, low potency active substances will not have

unnecessarily tight controls, and each active substance will be considered individually, but they are time consuming and have greater complexity. Whichever approach is taken it should be based upon a risk assessment. Setting specifications based upon the analytical capability should not be adopted; that is, excessively low limits should not be set just because the analytical method can achieve it, but equally too high a limit should not be accepted because of inadequate methods, said Mr Wood.

Leachables are chemicals that migrate from packaging into medicines under normal use, said Arthur J. Shaw, of Pfizer, US. Improvements in analytical technology have allowed the detection of trace-level leachables coming from materials used to contain and package pharmaceutical drug products. Although many leachables have been shown to pose no significant health risk to patients, the job of detection, identification, quantification and safety qualification of leachables must be undertaken to assure patient safety. Dr Shaw reported a

case-study carried out by the Product Quality Research Institute to determine if justifiable thresholds could be established beneath which detection and reporting of leachables could be suspended. The team focused on a narrow category of inhalation drug products and determined that such a threshold could be drawn. The results showed that a safety concern threshold of 0.15 µg/day total daily intake for identification and reporting of leachables was suitable for inhalation drug products. An analytical process for the detection and measurement of leachables present in a drug product was also developed that met the team's goal for practicality and scientific standards. The process consisted of forced solvent extractions of all the packaging components that may be able to release compounds (extractables) into the drug product followed by identification and semi-quantification, if above the safety concentration threshold. Methods capable of detection and measurement of these extractables, in the presence of drug product, would then be developed and validated.