

New developments in chromatographic and other analytical techniques

Nicholas D. Wood reports from a seminar for those involved in analysis or development of medicines, medical devices or their components

Andrew Baker, from AstraZeneca global process research and development, discussed his company's system for the rapid development of capillary gas chromatography (GC) methods within the pharmaceutical industry.

The common myth that GC is not applicable to drug substances — because these have high boiling and melting points, are often salts and are relatively unstable — has been dispelled, he said. Hence, there has been a renaissance of GC at AstraZeneca. Stability is improved by use of suitable media for non-ionised samples, an appropriate temperature in the injection headspace (where the sample is volatilised before it enters the chromatographic column), and deactivated silica particles in the column.

Selecting one from a short range of pre-set GC methods during development saves time and enhances convenience because:

- These are rapid procedures, often taking less than 10 minutes' analysis time, using shorter columns than usual
- These use existing equipment and predefined settings
- Standard methods can be operated by any member of AstraZeneca staff, including those in process development as well as quality assurance/control, so further training is unnecessary
- These give adequate results for more than 95 per cent of pharmaceutical components, including drug substances, intermediates, by-products or degradants

Only if molecules warrant further investigation, eg, by showing bioactivity, will full GC method development be undertaken.

Chromatography with MS

Nick Ordsmith, of Hall Analytical Laboratories, discussed the use of chromatography with mass spectroscopy in the structural elucidation of impurities and degradants.

Both MS and other common detector systems (eg, ultra-violet, flame ionisation, diode array) provide quantitative determination. However, MS also enables determination of molecules' structure (by degradation pattern) and relative molecular mass (RMM). These characteristics allow searching of electronic structural libraries to facilitate matching with known molecules.

The benefits of using a tandem approach eg, LC-MS-MS, or two different detection systems such as LC-UV-MS, have been investigated. This approach enables testing for a large variety of components in a sample, since one detector is unlikely to be able to analyse all molecules in a sample.

More recent MS methods, eg, matrix-assisted laser desorption ionisation time of flight (MALDI-TOF), enable high resolution results to be generated for less stable impurities.

Karl Fischer titration

Helga Hoffman, technical support manager at Sigma-Aldrich Laboratory, in her presentations on Karl Fischer (KF) titration, said that KF is a common method of determining water content extremely accurately and at low levels.

There is a stoichiometric chemical reaction between water, and a titrant that contains sulphur dioxide, alcohol (usually methanol), iodine (I₂) and an organic base. The presence of unreacted iodine defines the end of the reaction, she explained.

She described improvements to the method, including using coulometric rather than volumetric titration where appropriate, as well as replacement of pyridine (as base) or methanol, or both.

The KF titration endpoint was previously visual, with any remaining I₂ being detected by an indicator. A volumetric titration endpoint is now usually detected by measuring changes in conductivity across platinum electrodes, typically a sharp fall from 600mV to around 100–250mV.

If a suitable current passes through the reaction system, further I₂ is generated *in situ* and hence a titrant is not needed. This technique is termed "coulometry" (which also determines water content by conductivity changes) and has several advantages:

- Volumetric analysis can accurately detect water in milligram quantities but coulometric enables micrograms to be detected, enabling far smaller sample sizes.
- "Rezeroing" of the meters attached to the electrodes after a sample has been tested via coulometry enables the remaining liquid to be reused repeatedly. This saves money and time, and lessens solvent use, and hence has less ecological impact.
- Simultaneous control of pH to within the ideal and narrow range of 5–6. This improves accuracy, precision and speed of reaction while minimising side reactions.

The alkali traditionally used was pyridine, which is noxious, malodorous and often gives an imprecise endpoint. This has been replaced by imidazoles or other bases that give better defined results, are less toxic and slightly less offensive to the nose.

A variety of KF solvent systems have been developed to replace poisonous methanol. These include diethylene glycol monoethyl ether (improving reagent stability), ethanol (enabling ketones to be titrated) and hexanol or chloroform bases (often improve solubility).

Laboratory conditions have to be controlled. For example, air humidity will hydrate a sample or affect an ongoing KF test. To minimise this, transfer to reaction vessels is rapid and equipment is tightly closed. Molecular sieves maintain reagents in an anhydrous state.

The addition of should be prompt, said Ms Hoffman, using a minimum of dead space and preventing ingress of air into the reaction vessel, said Ms Hoffman. Hence small-volume insulin syringes are used for many liquids, but viscous formulations are dripped from a wide-bore needleless syringe through an entry port with a sealed flange.

The cathode blackens with time, becoming coated in partially oxidised sulphur compounds. This can be cleaned with an alcohol or, if necessary, concentrated nitric acid. However, one of the most effective methods found to clean electrodes was toothpaste, she said.

Solid phase extraction

Lisa Fitzpatrick of Sigma Aldrich discussed optimising solid phase extraction (SPE). This technique is for materials that require pre-treatment if, for example, they are too dilute or too dirty, or if the sample matrix is incompatible with a column.

She described methods and tips showing how to optimise choice of SPE column, binders, inert supports, solvents and conditions using tricyclic antidepressants in plasma as an example.

Due to a high hydrophobic character these are strongly retained by reverse phase columns, and this is enhanced further by use of a high pH solvent. For this reason, she explained, thorough washing, in order to remove salts, sugars and proteins is required. After that, the addition of an organic solvent phase of typically up to 80 per cent methanol enables elution of the tricyclic antidepressants and their metabolites from the solid phase.