TDM by LC/MS-Single Analyte, Multiplexing and Novel Drugs

Gareth Hammond
Technical Manager, Clinical Scientist Operations, Waters Corporation
United Kingdom

Scope of the lecture:
The scope of the lecture is the use of liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the quantification of Therapeutic Drugs. The subject matter will be covered using a timeline from the initial beginnings of LC-MS/MS for the quantification of immunosuppressive drugs through to its expansion to quantify different TDM drugs and multiplexing. The final section will look to the future and how LC-MS/MS quantitative assays will be developed during clinical trials of personalized medicines including monoclonal antibodies and modern therapeutics.

Learning objectives:
1. Why Therapeutic Drug Monitoring?
2. Why LC-MS/MS has been widely adopted in clinical laboratories to quantify analytes and manage patients requiring TDM and what assays are currently available.
3. What is the future for TDM and LC-MS/MS?

Extended abstract:
1) Why Therapeutic Drug Monitoring?

The International Association for Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) adopted the following definition:

> "TDM is a multi-disciplinary clinical specialty aimed at improving patient care by individually adjusting the dose of drugs for which clinical experience or clinical trials have shown it improved outcome in the general or special populations. It can be based on a *a priori* pharmacogenetic, demographic and clinical information, and/or on the *a posteriori* measurement of blood concentrations of drugs (pharmacokinetic monitoring) and/or biomarkers (pharmacodynamic monitoring)."

The criteria for TDM are as follows:

- **Narrow therapeutic index/range**
  - Dose of drug which produced desired therapeutic conc. is close to dose that may produce toxic serum conc.
- **No clearly defined parameter that allows dose changes**
  - Toxicity due to poorly defined endpoint if the drug is not monitored
- **Unpredictable relationship between dose/clinical outcome**
  - Efficacious dose in one patient may cause toxicity in another patient
- **Drugs with nonlinear PK parameters are candidates**
  - Small change in dose may cause disproportionate conc. increase (toxic)
  - Accumulation of drug may cause toxicity due to drug-drug interaction
- **Toxicity may cause hospitalization, organ damage, death**
- **Correlation between serum conc. and efficacy/toxicity**

2) Why LC-MS/MS has been widely adopted in clinical laboratories to quantify analytes and manage patients requiring TDM and what assays are currently available.

The clinical chemist has always looked to analytical advancements to address analytical deficiencies in the clinical laboratory. Analytical techniques employed in the laboratory have included radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELIZA), gas chromatography with various detectors and HPLC-UV amongst others.

Towards the end of the 20th century clinical chemists first started to build on their LC-UV knowledge and investigate LC-MS/MS as a means to address the needs of the clinical laboratory in quantifying analytes, The measurement of immunosuppressive drugs as an aid in the management of patients following solid organ transplant was the first TDM assay to be tackled with the first quantitative LC-MS/MS assays being developed for the individual quantification of immunosuppressive drugs cyclosporin and tacrolimus (FK506).
For the immunosuppressive drugs, the combination of LC and MS is able to provide chromatographic separation and mass selectivity to resolve assay challenges such as sensitivity, matrix interference, recovery and linearity. The basic steps to a successful LC-MS/MS method include:

- Sample preparation (initial sample clean-up prior to injection on to a chromatographic column)
- Gradient elution of the analyte off the chromatographic column away from regions of interference
- LC eluent is introduced into the MS where the analyte is ionized (precursor ion), collisionally dissociated to produce specific fragment ions (product ions) and detected

Blanks, calibration standards, quality controls and samples are prepared in batches and analysed. The blanks and quality controls are for batch control and the calibration standards are used to create a concentration versus response curve from which the concentration of unknown sample responses can be back-calculated.

Since the development of the first LC-MS/MS assays, the laboratory has looked to improve throughput, workflow and the return on investment for instruments which require large capital outlay. Clinical chemists looked to free time on the LC-MS/MS by:

- Decreasing analytical runtimes and injection-to-injection
- Quantify more than one analyte at a time (e.g. tacrolimus, cyclosporine, sirolimus and everolimus in a single analysis)
- Develop assays for more analytes

The choice of analyte has often been driven by the need for sensitivity and the challenge of assays where there traditionally has been no immunoassay such as anti-epileptics or newer drugs.
Some of the challenges which have now been answered by LC-MS/MS are:

- Anti-epileptic panels
- Azole antifungal panels
- Oncology drugs
- Antibiotics
- HIV drugs
- Pain management
- Monoclonal antibodies

The ability to quantify many of the analytes and panels above has only been possible through advancements in LC and MS technologies as well as software and connectivity to laboratory informatics management systems (LIMS). UPLC developments and new column technologies have seen improvements in throughput and performance for analytes whilst MS developments including ionization efficiency, ion optics, detector and electronics have improved detection and sensitivity.

3) What is the future for TDM and LC-MS/MS?

TDM forms the foundation of personalized medicine by characterizing sources of variability in drug disposition and response to individualize drug dosing. It has grown to include pharmacogenomic and other biomarker-driven strategies for patient segmentation. There are currently over five hundred therapeutics in development. What can LC-MS/MS bring to this area?