# Mass spectrometry: the gold standard in clinical routine

The application of mass spectrometry has evolved considerably since its first use and mass spectrometric methods were initially introduced in laboratory medicine approximately 40 years ago [1]. The very recent popularity of clinical mass spectrometry can be attributed to the high specificity, accuracy and reliability due to the direct analysis of ions without the risk of cross reactivity as described for antibody detection in immunoassays [2] as well as the ability to detect multi-analytes in a single run. Initially, GC-MS was used for biological analysis, however, this method requires volatile analytes, demanding extensive extraction and derivatization steps for nonvolatile and thermally unstable compounds typically found in clinical analysis. This is not particularly attractive in a clinical setting, in contrast to LC-MS/MS which offers the advantages of mass spectrometry analysis in combination with a simpler sample preparation technique.

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One such example is the analysis of methylmalonic acid (MMA), an important biomarker for the identification of vitamin $B_{12}$ deficiency which, if left untreated, can lead in the long term to permanent neurological damage and/or to hematological and gastroenterological diseases. The sole determination of holoTC, the active form of vitamin $B_{12}$, does not have the same diagnostic significance as the combined measurement of holoTC and MMA, as the MMA concentration shows a possible vitamin $B_{12}$ deficiency even before the actual vitamin level decreases [3]. Traditionally, the reference method for this parameter in plasma/serum is GC-MS which, as mentioned above, requires an extremely complex sample preparation that can take several hours [4]. In contrast to this, the sample preparation for LC-MS/MS from Chromsystems is much easier, and, with just a few minutes processing time, considerably faster, while requiring only one quarter of the sample material (see table 1).

***Mass**Chrom* MMA KIT

**SAMPLE PREPARATION FOR PLASMA/SERUM**

- Place 100 µl of sample/calibrator/control into Clean-Up tubes.
- Add 50 µl of Internal Standard and mix briefly (vortex).
- Centrifuge for 10 min at 14000 x g.
- Transfer the filtrate to an autosampler vial.
- Inject 10 to 20 µl of the filtrate into the LC-MS/MS system.

**SAMPLE PREPARATION FOR URINE**

- Pipette 200 µl sample/calibrator/control in transparent reaction vials.
- Add 25 µl Internal Standard and mix 10 s (vortex).
- Dilute sample with Dilution Buffer in an autosampler vial.

*Table 1: Sample preparation procedure for the determination of MMA concentrations by LC-MS/MS using the Chromsystems **Mass**Chrom MMA kit [4].*

Furthermore, data from plasma and urine MMA determinations by the reference GC-MS method and the new LC-MS/MS technology show a strong correlation and excellent agreement (Fig. 1). Therefore, the described LC-MS/MS technique represents a fast, reliable and robust method for routine analysis, achieving a higher throughput and higher efficiency.

## Sample preparation as a pivotal step

The correct analytical procedure from extraction and sample preparation, through to the chromatography and MS setup is a prerequisite to achieve optimal results by mass spectrometry, and to fulfil the requirements in clinical diagnostics. The development of an appropriate sample preparation procedure can be complicated and time-consuming, requiring considerable work in order to sensibly embed it in the overall analytical procedure. The ultimate goal is the enrichment of the molecule of interest by a simultaneous elimination of compounds that cause ion suppression or enhancement effects. Moreover, components from plastic, chemicals like salts or particularly from the human matrix (whole blood, serum, plasma, urine), potentially co-eluting from the LC system can compete with the analytes during the ionization process. This leads to a change in compound ionization, and consequently alters the MS signal at the detector [5]. This process is called "ion suppression" and Bonfiglio *et al* [6] systematically analysed these effects and have found not surprisingly that they are dependent on the sample preparation technique used as well as the compound to be analysed. More polar analytes also showed stronger effects than less polar ones. Short-term variations in ionization can also compromise the accuracy of analyses, if the method is not sufficiently robust. If these variations have a differential impact on the target analyte and internal standard, the overall analysis is affected [7]. The authors also concluded the need for calibration material to be as similar as possible to the sample matrix. In addition, the choice of an appropriate internal standard helps to reduce matrix effects; whenever possible, an isotopically labelled version of the analyte is the ideal choice.

Depending on sample specimens and analyte characteristics, sample preparation techniques can encompass liquid-liquid extraction, solid phase extraction or protein precipitation and are also crucial for the removal of materials that may contaminate the column, trap-column or the analytical system.

Considering all of these factors, successful method development where all parameters work well within at least acceptable levels of CVs, recovery and appropriate

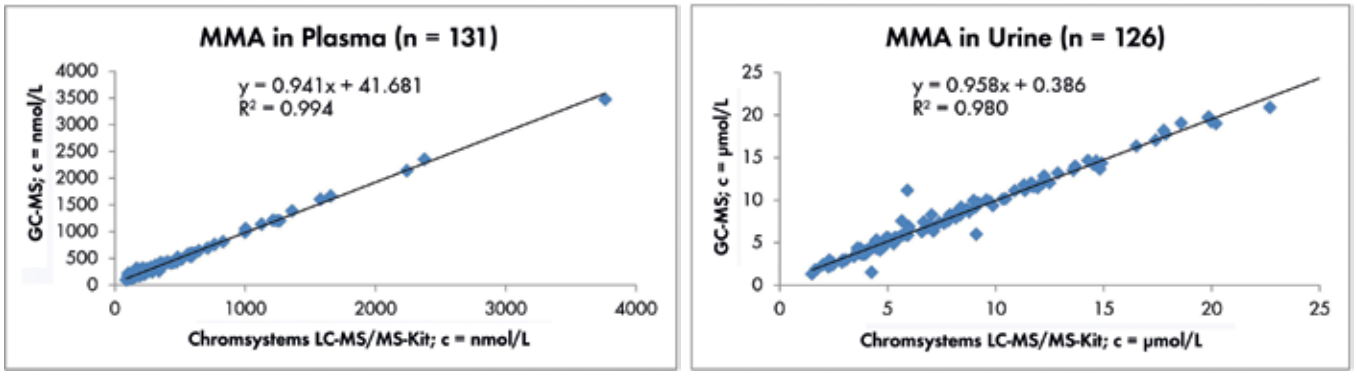


*Figure 1. The analyses of MMA from plasma (n=131) and urine (n=126) run on the LC-MS/MS system from AB SCIEX (AB SCIEX 4500) were compared with the GC-MS reference method and show a high correlation.*

limits of quantification (LOQs) can be very challenging. Furthermore, full establishment of a method that is comprehensively validated in the laboratory is a laborious process. The use of commercially available kits, like the one mentioned above for MMA, which have gone through numerous optimization, verification and validation processes from sample preparation through to MS analysis represents a secure, robust and time-saving alternative for clinical laboratories.

## Multi-analyte determination

The capability of LC-MS/MS systems for the analysis of several compounds in a single run sounds efficient and relevant, e.g. for the simultaneous analysis of drugs and their metabolites, but may not be as easy as it seems. Every single analyte in a patient sample may possess different chemical and physical properties that affect its recovery in the sample preparation procedure. Consequently, some compounds may be extracted more efficiently than others. Therefore, it can be a highly complex task with a significant amount of work to develop a general sample preparation procedure for quantification of numerous drugs and metabolites, with many of them being analysed in a single run (see Fig. 2), aimed at simplifying the laboratory workflow.

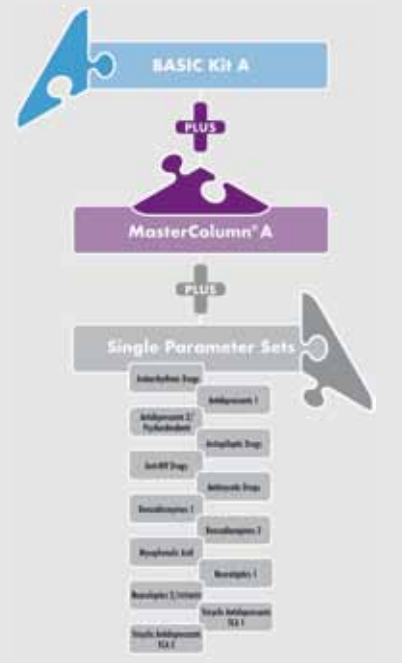


*Figure 2. Chromsystems offers a multi-analyte system for determination of more than 150 drugs and metabolites. It consists of 3 modules: the Basic Kit A with mobile phases and reagents, the MasterColumn A for all determinations and the parameter sets.*

## Automation for a higher throughput

One of the major challenges clinical laboratories have been facing is the simplification and acceleration of sample preparation for LC-MS/MS. By using an automated workflow potential pipetting errors can be minimized and, in parallel, the throughput can be drastically increased. This is relevant, for example, to large transplant centres that analyse a high number of patient samples for immunosuppressive drugs, but nevertheless need to achieve fast and reliable results by LC-MS/MS. To date, there is only one system on the market (MassSTAR) that allows a fully automated CE-IVD workflow for immunosuppressants including sample tracking, LIMS connectivity and clotting detection. The automated method offers a time saving of approximately 80% compared to manual preparation. A comparison between manual and automated sample preparation and measurement techniques for the four immunosuppressants cyclosporine A, everolimus, sirolimus and tacrolimus showed very high correlations (Fig. 3). Automated and manual preparation procedures therefore produce almost the same results, with automation reducing the time needed for sample extraction while also increasing sample throughput. These automation options are also provided by Chromsystems for other parameters, such as vitamin $D_3$/$D_2$, the immunosuppressant mycophenolic acid and antiepileptics, for which comparable correlations between the manual and the automated methods have also been shown.

## A gold standard in routine

LC-MS/MS is a valuable technique that is often used in reference methods for a wide range of parameters. Its main drivers for growth in clinical laboratories are the limitations of immunoassays for low molecular weight compounds, the easier workflows and higher throughput [8]. However, there are certain downfalls that need to be addressed with one of the most, or even the most critical factor in clinical mass spectrometry being the application of an appropriate sample preparation procedure that is robust as well as reliably fulfilling analytical requirements. A number of proven and CE-IVD approved LC-MS/MS kits for sample preparation from Chromsystems are available and simplify the workflow in the laboratory. Furthermore, automation is also possible for a range of parameters, reducing hands-on time and increasing throughput for those laboratories with the need for higher throughput.

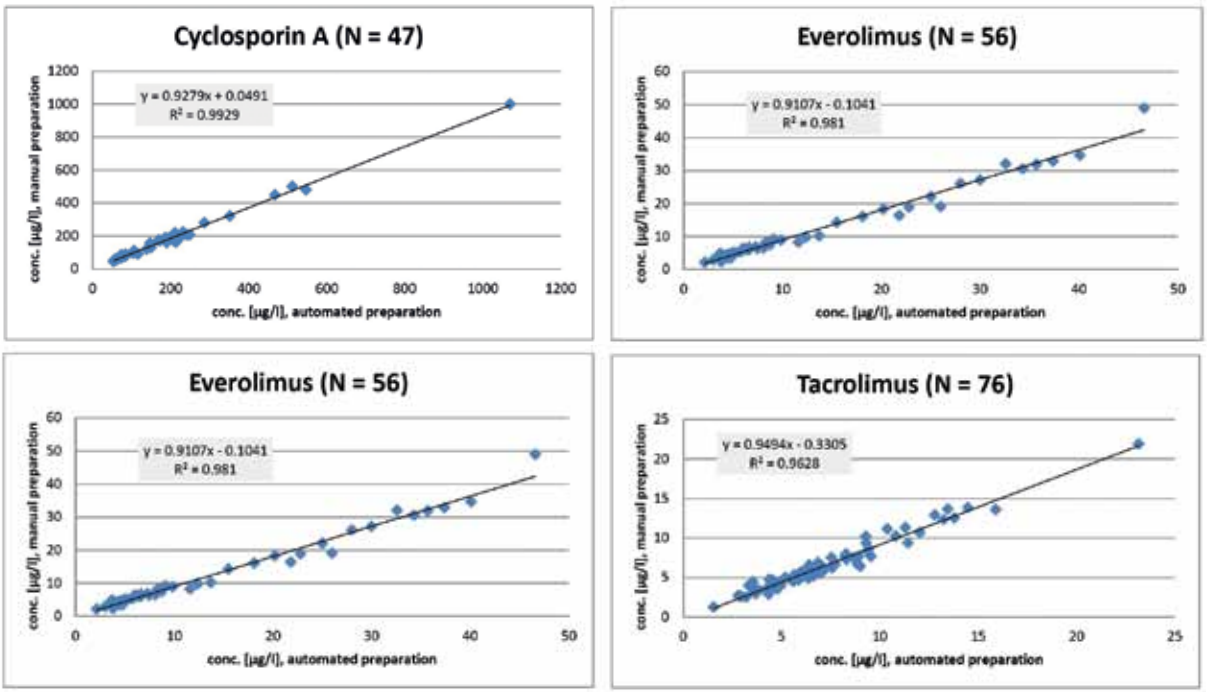


*Figure 3. Patient samples were prepared manually and by automation and analysed for the four listed immunosuppressants cyclosporine A, everolimus, sirolimus and tacrolimus. A high correlation is shown between the results with and without automation of the sample preparation.*

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