# Automation and Liquid Chromatography-Tandem Mass Spectrometry in Therapeutic Drug Monitoring

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Therapeutic drug monitoring (TDM) has become a key clinical tool to help individualise therapy, check compliance and maximise response while lowering side effects. Liquid chromatography-tandem mass spectrometry has become a major technology in TDM given its inherent specificity, sensitivity and quantitative capability for small molecule drug analysis. Within the context of a routine clinical pathology environment there are considerable advantages in integrating mass spectrometry into small molecule drug monitoring when compared to immunoassays. This review considers the impact of LC-MS/MS in a routine clinical pathology laboratory compared to conventional immunoassay techniques and highlights mass spectrometry in the analysis of immunosuppressant's and anticonvulsants.

## 1. Introduction

Therapeutic drug monitoring (TDM) is a multi-disciplinary science helping to understand the factors that determine the dose-effect relationship and to use this knowledge to optimise drug treatment (maximise efficacy /minimise side effects). In many routine clinical pathology laboratories, the panels of drugs subject to routine TDM in patients is limited, including several immunosuppressive drugs, antibiotics, antiepileptics, antidepressants, digoxin and methotrexate. This reflects the need to monitor drug classes that have a narrow therapeutic index, established consequences for under- or over-dosing, a defined relationship between blood concentration and clinical/toxic effect, significant variation within and between individuals and for drugs that have a proven knowledge base for clinical management [1-3] (see *Table 1*).

In most routine clinical pathology laboratories, automated immunoassay platforms dominate bioanalytical drug assays. However, immunoassay techniques may produce results that have a bias due to the cross reactivity of the active metabolites, batch-to-batch heterogeneity in antibodies or reagent quality, high-dose-hook effect and, for some drug immunoassays, a high cost per analysis [4].

As precision medicine emerges as a possible approach to treat a specific individual patient with a specific disease taking into account individual variability in genes, environment and lifestyle TDM is likely to have a high impact in dose adjustments. However, this is a novel application for TDM and requires extensive assay development and validation together with rapid turnaround times so that assays can be used for drug development and individual patient care. Supporting such analytical and clinical strategies requires methodologies which are versatile and can be easily adapted to a 'laboratory-developed test' (LDT) or 'in-house' assay.

In this article, we highlight the application of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) widely regarded as the gold standard in TDM for immunosuppressants and anticonvulsants and considers the development of further automation.

## 2. LC-MS/MS for TDM

From a historical point of view, liquid chromatography techniques have been used for TDM with ultra-violet (UV), photo diode array (PDA) or fluorescent detection (FLD) systems for several decades. However, despite the high impact of ultra-high-performance liquid chromatography (UHPLC) [5-7] in reducing run times and enhancing separation efficiency, such detection techniques are limited in terms of specificity often resulting in extensive sample cycle times and poor sensitivity as many compounds lack a natural chromophore or fluorophore [1]. More recently mass spectrometry is now regarded as a key technique for routine clinical pathology laboratories delivering robust, rugged platforms with highly selective and sensitive detection [8]. Its capability in the development of assays for individual drugs ('laboratory-developed tests' (LDTs) or 'in-house' assays) and in multiplexing analyte panels creates new opportunities in expanding the number of drug assays. By increasing the number of drug assays, helping to provide better access to the technology and using novel blood sampling strategies including micro-sampling for pediatric TDM or at home sampling [9-12] also helps position TDM for personalised medicine.

## 3. Automated Sample Management and Preparation

Biological fluids are highly complex matrices which present challenges in matrix management as endogenous and exogenous components result in compound and system-specific effects in mass spectrometry. Negating the effects of the matrix needs to be carefully considered in all sample preparation and management protocols. Matrix effects can lead to isobaric interferences, particulate clogging, ion suppression or ion enhancement resulting in a difference between the signal intensity detected in a neat standard solution compared to a matrix-matched standard [13-16].

In electrospray ionisation, ion suppression is due to a change in the droplet formation and surface tension which will affect charge transfer efficiency. Non-volatile compounds such as blood phospholipids, salts, uncharged matrix components, reagent impurities, drugs and metabolites are known to produce ion suppression or enhancement [17]. To help reduce the impact of matrix effects on bioanalytical assays there are several strategies open for the analyst. One of the most important techniques is to use appropriate and validated internal standards, particularly stable-isotope-labelled analogues (SIL-ISTD) to help correct for ion signal changes and handling errors in sampling preparation protocols. However, for many assays the effect of high inter and intra-patient variability in endogenous molecule concentrations also requires sample clean-up using extraction or purification techniques such as liquid-liquid extraction (LLE), solid-phase extraction (SPE) or protein precipitation (PPT). Each technique needs to be considered in the context of assay performance to achieve an acceptable level of accuracy and precision while also taking into account the efficiency and recovery of the extraction in addition to the ease of use and cost per sample.

*Figure 1: General view of the LC-MS/MS system with integrated sample preparation module CLAM-2000.*

For high throughput assays, automated sample preparation platforms are now used. One such example is the Clinical Laboratory Automated sample preparation Module (CLAM-2000) integrated with LC-MS/MS (LCMS-8060; *Figure 1*). The CLAM-2000/LCMS-8060 platform is the first of its kind in bringing an automated solution to routine clinical pathology accelerating the concept of patient sample to result. The CLAM-2000 supports integrated calibrators and quality controls throughout the batch analysis, parallel analysis and sample preparation and can be fully adapted to a range of sample preparation protocols including reagent aliquoting, ISTD addition and extraction for automated LC-MS/MS analysis.

The system is designed as an open architecture for method development and routine sample analysis enabling the validation of LDT's to increase sample throughput, reduce the risk of human errors, minimise user contact with biological samples and simplify operation.

Its application to immunosuppressant's and anticonvulsant's is highlighted below.

## 4. Immunosuppressant drugs

Immunosuppressant drugs are used to reduce the activity of the immune system and prevent transplant rejection. The major drugs used are calcineurin inhibitors, cyclosporine and tacrolimus, the mTOR inhibitors, sirolimus and everolimus. Circulating concentrations of these compounds should remain within a narrow therapeutic range, as overdosing can cause serious toxicity and long-term morbidity, and underdosing can cause rejection [3]. As immunosuppressant drugs result in a high pharmacokinetic variability between individual patients, TDM is now an established approach to mitigate the risks associated with organ transplantation.

Several commercial immunoassays are available for the TDM of immunosuppressant's, however, all immunoassays show a significant positive bias compared to LC-MS/MS methods [18]. Despite the availability of automated immunoassays each test is restricted to one analyte for each test when in many clinical settings multiple immunosuppressants are used in one individual patient [1, 19]. In this example, an automated LC-MS/MS method is described for the routine TDM analysis of immunosuppressants.

### 4.1. Materials and Methods

The quantitative analysis of immunosuppressant drugs was performed using reagents provided in a Dosimmune® kit (Asachim, France) [20]. A UHPLC-MS/MS system (Nexera X2 and LCMS-8050, Shimadzu Corporation, Kyoto) within online SPE was used for sample analysis (see *Figure 2*). Automatic sample preparation was performed using the CLAM-2000 module (Shimadzu Corporaiton, Kyoto). Sample preparation was performed using the extraction buffer and internal standard set provided in the kit.

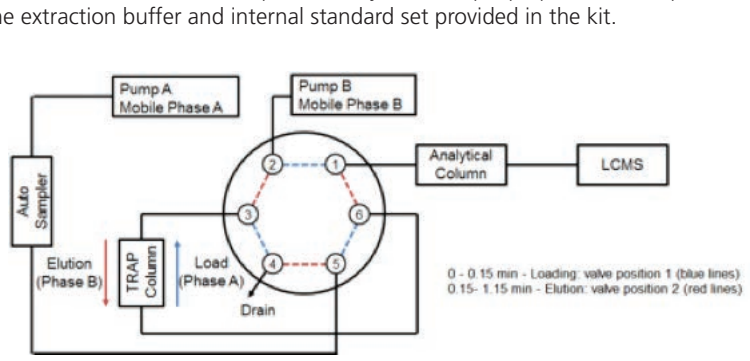


*Figure 2: Flow diagram of online SPE-LC-MS/MS system for immunosuppressants.*

25 µL of whole blood (calibrators, quality control samples (QC) or sample) were mixed with 12.5 µL of SIL-ISTD solution and 175 µL of extraction buffer (mixture of zinc sulphate 0.1M, methanol and acetonitrile 5/3/2 v/v). After 30 seconds of vortex, the samples were filtered for 1 min with the CLAM-2000 device. The resulting extract was then automatically transferred into the LC-MS/MS autosampler for analysis. Then, 20µL of extract was injected onto the online SPE column (Ascentis C8 5µm 30x4.6mm, Supelco, USA). After 0.15 min, the SPE column was backflushed and the resulting analytes transferred to the LC column (Ascentis C18 5µm 50x2.1mm, Supelco, USA), maintained at 65°C, for separation and detection. The SPE mobile phase (formate buffer/methanol 9/1 v/v) and LC mobile phase (formate buffer/methanol 1/9 v/v) were pumped at 2 mL/min and 0.8 mL/min, respectively. An overview of the analytical process is shown in *Figure 3*. Using sample preparation overlapped with analysis, a result was generated every 4 minutes. Mass spectrometry parameters are described in *Table 2*. As it is common for these compounds, the monitored transitions used ammonium adducts $[M+NH4]^+$ as the precursor ion.

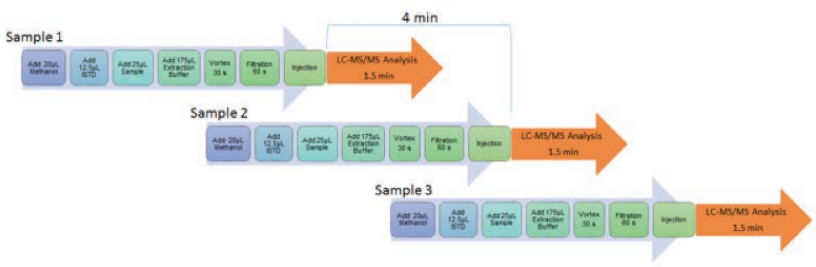


*Figure 3: Sample processing overview for immunosuppressants.*

Calibration standards and QC samples prepared in whole blood provided with the kit were used to assess data quality. Calibration (6 levels) ranged from 0.5 to 40 ng/mL for everolimus, sirolimus and tacrolimus, and from 5 to 1500 ng/mL for cyclosporin A. Four QC levels were processed in 8 individual replicates.

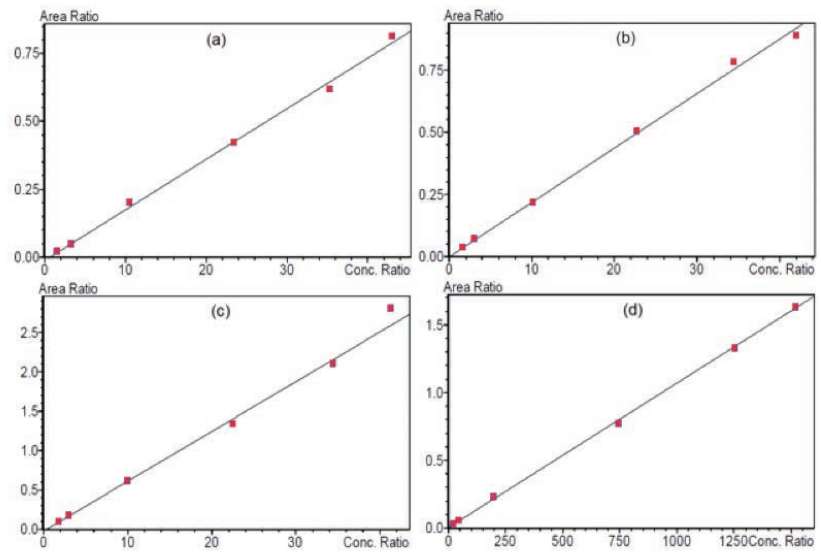


*Figure 4: Calibration curves for (a) Everolimus, (b) Sirolimus, (c) Tacrolimus and (d) Cyclosporine A.*

### 4.2. Linearity

Linearity of the method was assessed by calculating the relative deviation of calibration standards against the calculated linear regression model. In all cases, deviation was within ±15%, meeting the acceptance criteria. Typical calibration curves are showed in *Figure 4*.

### 4.3. Accuracy and Precision

Accuracy and precision of the QC samples were calculated across the 8 replicates per concentration level. Results are reported in *Table 3*. A typical chromatogram of target compounds is shown in *Figure 5*. All QC analyses were within the acceptance criteria for accuracy and precision.

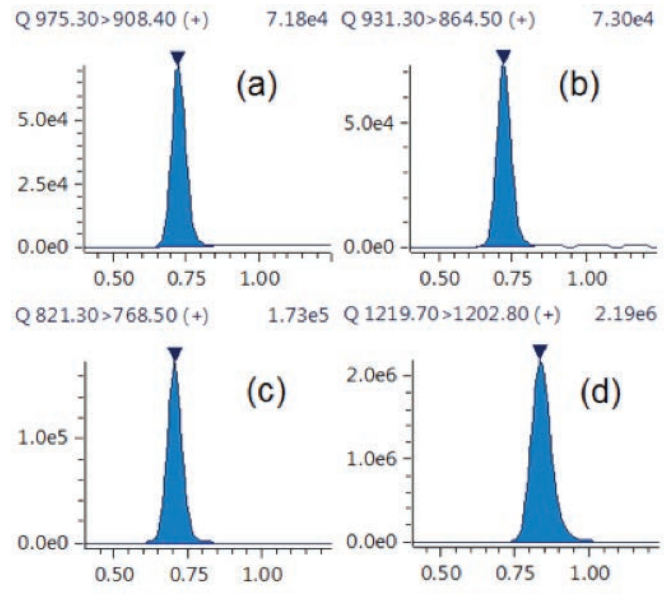


*Figure 5: Middle level QC chromatogram for (a) Everolimus, (b) Sirolimus, (c) Tacrolimus and (d) Cyclosporine A.*

### 4.4. Comparison with immunoassay

Patient samples were assayed in parallel by immunoassays and LC-MS/MS (see *Figure 6*, data not published, courtesy of Shimadzu Italy). For each drug compound, there was good agreement between both techniques with a correlation coefficient r>0.9.

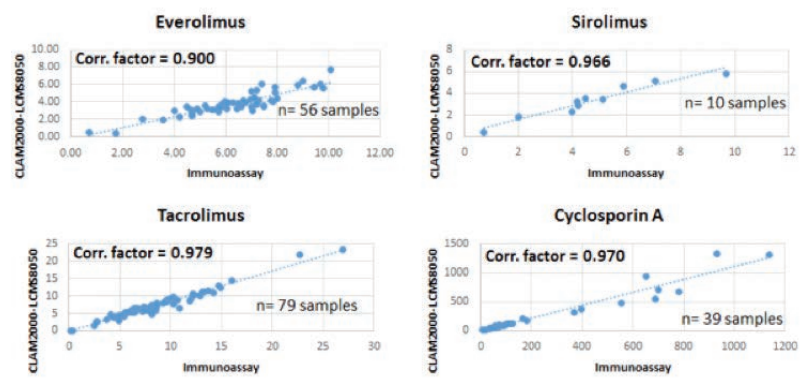


*Figure 6: Correlation data between immunoassay and LC-MS/MS for immunosuppressants.*

## 5. Anticonvulsants

Epilepsy is a chronic neurological disorder which is characterised by recurrent epileptic seizures whose frequency and rhythm are difficult to predict. For the pharmacological therapy of epilepsy, a variety of antiepileptic drugs (AEDs) are available today, most of which exhibit a pronounced intra- and inter- individual variability in pharmacokinetics. In many patients, it is necessary to use multiple drugs, however, as interactions between different AEDs also affect the pharmacokinetics there is a clear need for TDM [21].

AEDs as a therapeutic class show diverse chemistries because of their different mode of action. In addition, some benzodiazepines are also used in the treatment of seizures. However, the therapeutic concentrations are in lower ranges compared to most AEDs. Structures of representative compounds are presented in *Figure 7*.

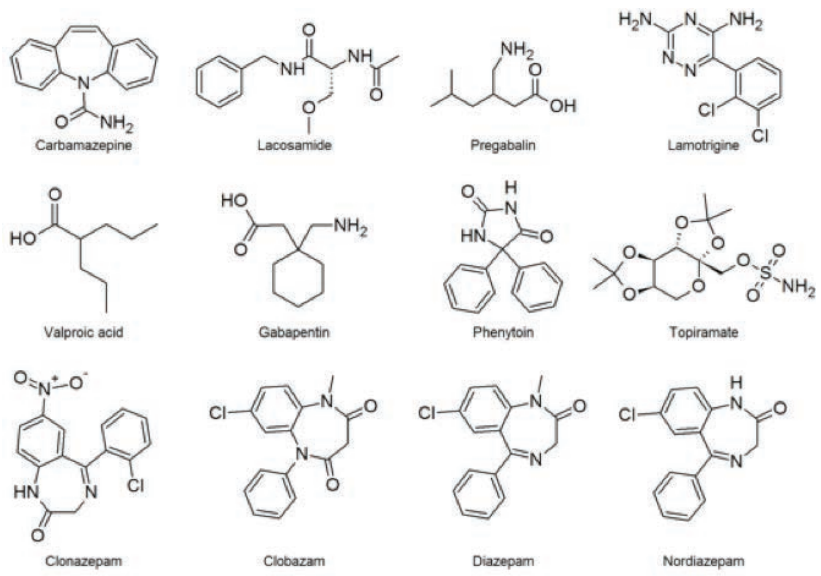


*Figure 7: Structures of the most common antiepileptic drugs and benzodiazepines included in this study.*

To meet this need a TDM method was developed to measure simultaneously a large panel of anticonvulsant drugs with different chemistries and target concentrations.

## 5.1. Materials and Methods

Certified standard solutions of each compound were purchased from Cerilliant (Sigma-Aldrich, USA). $^{13}C_6$-Zonisamide and $D_5$-Phenobarbital were used as the internal standard in positive and negative ionisation, respectively. The *Table 4* summarises the list of compounds assayed.

Calibration standard levels were prepared by spiking a blank plasma pool (EDTA-K3, 6 donors, mixed gender, BioreclamationIVT, USA). Seven levels were prepared. For each compound, the calibration range was determined using the therapeutic reference range.

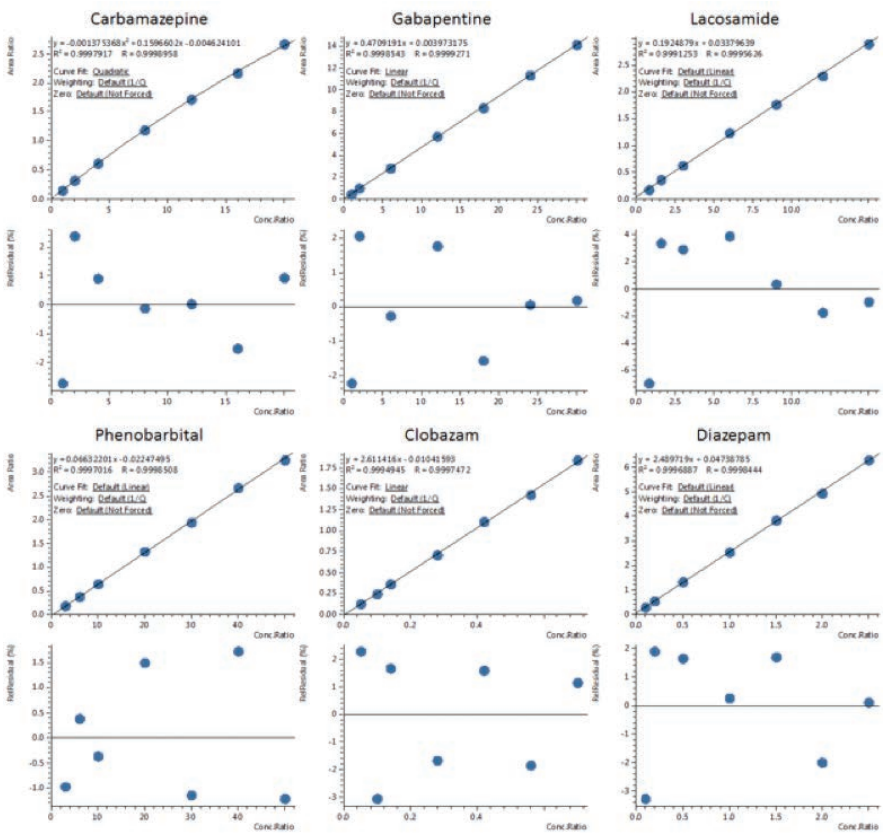


*Figure 8: Representative calibration curves of anticonvulsants.*

The lower limit of quantification (LLOQ) was set as 5 times lower than the low reference concentration. The upper limit of quantification (ULOQ) was set as 1.5 times higher than the high reference concentration. Targeted calibration ranges can be found in *Table 4*.

Quality control samples were prepared. Concentration levels were LLOQ (QCLOQ), 3 times the LLOQ (QC A), 50% of the concentration range (QC B) and 90% of the concentration range (QC C). Five individual replicates were prepared per level.

Automatic sample preparation was performed using CLAM-2000 module (Shimadzu, Kyoto) coupled to UHPLC-MS/MS system (Nexera X2 and LCMS-8050, Shimadzu, Kyoto).

For sample preparation, 30 µL of plasma were mixed with 270 µL of SIL-ISTD solution (10 µg/mL in methanol). After 30 seconds of vortex, the samples were filtered during 1 min. The resulting extract was then automatically transferred into the LC-MS/MS autosampler for analysis. Then, 0.5µL of extract were injected on the LC column (Shim-Pack GIS C18 5µm 50x2.1mm Shimadzu, Japan), maintained at 45°C. Mobile phase were (A) ammonium formate buffer 3mM pH3.6 and (B) methanol. A gradient from 10%B to 90%B in 2 minutes was run at 0.6 mL/min. The total run time was of 3.5 minutes. Two transitions per compound were acquired for quantification and confirmation of identification (except for Valproic Acid). Mass spectrometry parameters are described in *Tables 5 and 6*.

## 5.2. Linearity

Linearity of the method was assessed by calculating the relative deviation of calibration standards against the calculated linear regression model. In all cases, deviation was within ±15%, meeting the acceptance criteria. Typical calibration curves are shown in *Figure 8*.

## 5.3 Recovery

Recovery of the method was evaluated by comparing peak areas measured in QC samples prepared in plasma (n=6) to the ones measured in QC samples made in neat solution (n=5). Total recovery, combining extraction and matrix effect, was measured. Results are presented in *Table 7*. For all compounds, recovery was consistent across the concentration range and the average was greater than 80%.

## 5.3. Accuracy and Precision

Accuracy and precision of the QC samples were calculated across 5 replicates per concentration level. Results are reported in *Table 8*. A typical chromatogram of target compounds is shown in *Figure 9*. All QC samples were within the acceptance criteria for accuracy and precision.

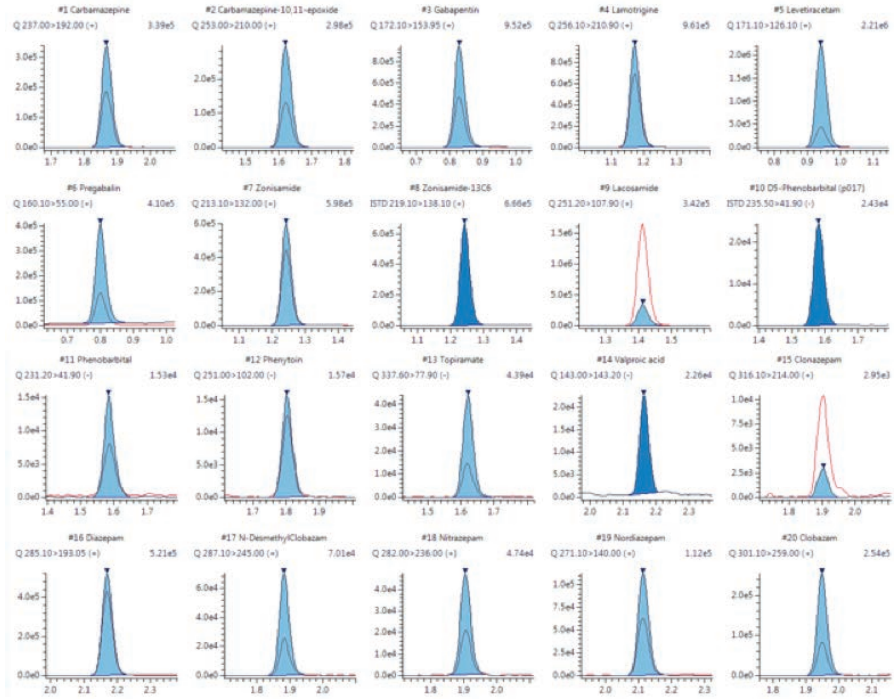

*Figure 9: Representative anticonvulsant chromatograms at the QC A level (3 times the LLOQ).*

# Tables

***Table 1: Targeted drugs in TDM***

| Class | Drugs |
|---|---|
| Antibiotics | Amikacin, Amoxicillin, Azithromycin, Cefixime, Cefoperazone, Cefuroxime, Cephalexin, Clarithromycin, Clavulanic acid, Clindamycin, Colistin A and B, Daptomycin, Erythromycin, Ethambutol, Gentamicin, Isoniazid, Levofloxacin, Neomycin, Pyrazinamide, Rifampicin, Sublactam, Sulfamethoxazole, Tobramycin, Trimethoprim, Vancomycin. |
| Anticonvulsants | Carbamazepine, Clobazam, Clonazepam, Clorazepate, Diazepam, Eslicarbazepine acetate, Ethosuximide, Ezogabine (Retigabine), Felbamate, Gabapentin, Lacosamide, Lamotrigine, Levetiracetam, Lorazepam, Midazolam, Nitrazepam, Nordiazepam, Oxcarbazepine, Perampanel, Phenobarbital, Phenytoin, Pregabalin, Primidone, Progabide, Rufinamide, Stiripentol, Tiagabine, Topiramate, Trimethadione, Valproic acid, Vigabatrin, Zonisamide. |
| Antidepressants | Amisulrpide, Aripiprazole, Citalopram, Clozapine, Doxepin, Duloxetine, Escitalopram, Fluoxetine, Flupentixol, Fluvoxamine, Haloperidol, Mianserin, Mirtazapine, Moclobemide, Nordoxepin, Norfluoxetine, Nortriptyline, Olanzapine, Paroxetine, Pimozide, Prochlorperazine, Quetiapine, Risperidone, Sertraline, Trazodone, Tryptophan, Venlafaxine, Zuclopenthixol. |
| Antifungals | Amphotericin B, Anidulafungin, Caspofungin, Fluconazole, Iodiconazole, Isavuconazole, Itraconazole, Micafungin, Posaconazole, Voriconazole. |
| Anticancer drugs | Busulfan, Carboplatin, Docetaxel, Erlotinib, Gefitinib, Ifosfamide, Imatinib, Irinotecan, Lapatinib, Lenalomide, Melphalan, Methotrexate, Nilotinib, Paclitaxel, Pemetrexed, Procarbazine, Raltitrexed, Sorafenib, Sunitinib, Tamoxifen, Tegafur, Thalidomide, Vinblastine, Vincristine. |
| Anti-viral drugs | Abacavir, Amprenavir, Atazanavir, Darunavir, Delavirdine, Didadosine, Efavirenz, Emtricitabine, Etravirine, Fosamprenavir, Ganciclovir, Indinavir, Lamivudine, Lopinavir, Maraviroc, Nelfinavir, Nevirapine, Raltegravir, Ribavirin, Ritonavir, Saquinavir, Stavudine, Tenofovir, Tipranavir, Valganciclovir, Viramidine, Zalcitabine, Zidovudine. |
| Cardioactive drugs | Acetyldigitoxin, Amiodarone, Deslanoside, Digitoxin, Digoxin, Methyldigoxin, Lanatoside, Rivaroxaban |
| Immunosuppressants | Cyclosporine, Everolimus, Mycophenolate glucuronide, Mycophenolic acid, Sirolimus, Tacrolimus. |

***Table 2: MS/MS parameters for immunosuppressants***

| Parameter | Value |
|---|---|
| **System** | : LCMS-8050 |
| **Ionisation** | : Heated ESI |
| **Acquisition Mode** | : MRM |

| **Transitions** | : Compound | MRM | CE (V) | Dwell time (ms) |
|---|---|---|---|---|
| | Everolimus | 975.6 > 908.5 | 20 | 59 |
| | $^{13}C_2D_4$-Everolimus | 981.5 > 914.5 | 20 | 59 |
| | Sirolimus | 931.6 > 864.5 | 18 | 59 |
| | $^{13}CD_3$-Sirolimus | 935.4 > 864.5 | 18 | 59 |
| | Tacrolimus | 821.5 > 768.6 | 22 | 59 |
| | $^{13}CD_4$-Tacrolimus | 826.4 > 773.6 | 22 | 59 |
| | Cyclosporin A | 1219.9 > 1202.8 | 21 | 59 |
| | $D_{12}$-Cyclosporin A | 1231.8 > 1214.9 | 21 | 59 |

| **Ion Source Temperature** | : Interface | Heating block | Desolvation line |
|---|---|---|---|
| | 200°C | 200°C | 250°C |
| **Ion Source Gas Flow** | : Heating gas | Drying gas | Nebulising gas |
| | 10 L/min | 10 L/min | 3 L/min |
| **CID Gas Pressure** | : 270 kPa | | |

***Table 3: Immunosuppressant QC Results***

| | Level (ng/mL) | %RSD | Accuracy |
|---|---|---|---|
| Cyclosporin A | Low (30.5) | 2.4% | 80.0% |
| | Mid-low (260.1) | 7.2% | 86.0% |
| | Mid-High (1172.8) | 3.5% | 93.6% |
| | High (1432.8) | 9.9% | 93.2% |
| Everolimus | Low (2.8) | 7.9% | 88.2% |
| | Mid-low (7.2) | 7.0% | 97.4% |
| | Mid-High (30.5) | 4.2% | 94.2% |
| | High (38.1) | 8.9% | 93.4% |
| Sirolimus | Low (3) | 15% | 87.0% |
| | Mid-low (7.5) | 8.0% | 93.6% |
| | Mid-High (29.5) | 5.4% | 96.0% |
| | High (36.6) | 11% | 96.5% |
| Tacrolimus | Low (2.7) | 9.1% | 99.5% |
| | Mid-low (6.9) | 5.4% | 97.0% |
| | Mid-High (28.9) | 3.2% | 99.0% |
| | High (36.2) | 3.9% | 98.0% |

***Table 4: Targeted anticonvulsant drugs***

| Compound Name | Acronym | Class | Calibration Range (mg/L) LLOQ | ULOQ |
|---|---|---|---|---|
| Carbamazepine | CBZ | Carboxamide | 1 | 20 |
| Carbamazepine-10,11-epoxide | EPO-CBZ | Carboxamide | 2 | 45 |
| Gabapentin | GBP | GABA analogue | 1 | 30 |
| Lacosamide | LCA | Modified amino acid | 0.8 | 15 |
| Lamotrigine | LMT | Triazine | 1 | 25 |
| Levetiracetam | LVT | Pyrrolidine | 4 | 70 |
| Phenobarbital | PBR | Barbiturate | 3 | 50 |
| Phenytoin | PNT | Hydantoin | 1 | 30 |
| Pregabalin | PGB | GABA analogue | 0.5 | 10 |
| Topiramate | TPA | Fructose derivative | 1 | 20 |
| Valproic acid | VPA | Fatty acid | 8 | 120 |
| Zonisamide | ZNA | Sulphonamide | 3 | 45 |
| Clobazam | CLBZ | Benzodiazepine | 0.05 | 0.7 |
| Clonazepam | CZP | Benzodiazepine | 0.005 | 0.07 |
| Diazepam | DIA | Benzodiazepine | 0.1 | 2.5 |
| N-Desmethylclobazam | DM-CLBZ | Benzodiazepine | 0.06 | 4.5 |
| Nitrazepam | NTZ | Benzodiazepine | 0.02 | 0.3 |
| Nordiazepam | NDIA | Benzodiazepine | 0.08 | 1.2 |

***Table 5: MS common parameters for anticonvulsants***

| Parameter | Value |
|---|---|
| **System** | : LCMS-8050 |
| **Ionisation** | : Heated ESI |
| **Acquisition Mode** | : MRM |

| **Ion Source Temperature** | : Interface | Heating block | Desolvation line |
|---|---|---|---|
| | 300°C | 400°C | 150°C |
| **Ion Source Gas Flow** | : Heating gas | Drying gas | Nebulising gas |
| | 10 L/min | 10 L/min | 3 L/min |

| **CID Gas Pressure** | : 270 kPa |
|---|---|
| **Pause Time** | : 1 ms |
| **Polarity Switching** | : 5ms |

*Table 6: MRM parameters for anticonvulsants*

| Name | Ionisation | Quantification MRM CE(V) | Confirmation MRM CE(V) | Dwell Time (ms) |
|---|---|---|---|---|
| **Carbamazepine** | pos | 237 > 192 -21 | 237 > 165 -43 | 8 |
| **Carbamazepine-10,11-Epoxide** | pos | 253 > 210 -12 | 253 > 167 -35 | 8 |
| **Gabapentin** | pos | 172 > 154 -15 | 172 > 137 -16 | 19 |
| **Lamotrigine** | pos | 256 > 211 -26 | 256 > 43 -53 | 14 |
| **Levetiracetam** | pos | 171 > 126 -15 | 171 > 69 -27 | 19 |
| **Pregabalin** | pos | 160 > 55 -22 | 160 > 97 -14 | 24 |
| **Zonisamide** | pos | 213 > 132 -14 | 213 > 77 -33 | 12 |
| **13C6-Zonisamide** | pos | 219 > 138 -15 | --- --- | 26 |
| **Lacosamide** | pos | 251 > 108 -20 | 251 > 91 -50 | 12 |
| **D5-Phenobarbital** | neg | 236 > 42 24 | --- --- | 18 |
| **Phenobarbital** | neg | 231 > 42 22 | 231 > 188 11 | 8 |
| **Phenytoin** | neg | 251 > 102 23 | 251 > 208 16 | 8 |
| **Topiramate** | neg | 338 > 78 31 | 338 > 96 25 | 8 |
| **Valproic Acid** | neg | 143 > 143 11 | --- --- | 20 |
| **Clonazepam** | pos | 316 > 214 -38 | 316 > 270 -25 | 8 |
| **Diazepam** | pos | 285 > 193 -31 | 285 > 154 -27 | 9 |
| **N-Desmethylclobazam** | pos | 287 > 245 -18 | 287 > 210 -31 | 8 |
| **Nitrazepam** | pos | 282 > 236 -24 | 282 > 180 -38 | 8 |
| **Nordiazepam** | pos | 271 > 140 -27 | 271 > 165 -27 | 9 |
| **Clobazam** | pos | 301 > 259 -21 | 301 > 224 -32 | 8 |

*Table 7: Recovery results for anticonvulsants*

| | | Total Recovery (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **CBZ** | **EPO-CBZ** | **GBP** | **LMT** | **LVT** | **PGB** | **ZNA** | **LCA** | **PBR** |
| **QC LOQ** | Pool A | 101 | 103 | 92.0 | 84.2 | 109 | 89.4 | 82.6 | 86.9 | 81.8 |
| | Pool B | 108 | 109 | 97.3 | 92.2 | 119 | 96.4 | 91.3 | 95.4 | 90.9 |
| **QC A** | Pool A | 98.7 | 103 | 91.9 | 83.9 | 107 | 90.0 | 82.2 | 88.1 | 83.0 |
| | Pool B | 101 | 104 | 91.8 | 85.9 | 109 | 90.9 | 84.8 | 90.4 | 77.5 |
| **QC B** | Pool A | 114 | 110 | 102 | 94.9 | 118 | 102 | 94.1 | 99.6 | 90.7 |
| | Pool B | 113 | 112 | 98.9 | 94.6 | 120 | 99.9 | 93.1 | 98.9 | 88.6 |
| **QC C** | Pool A | 104 | 105 | 94.3 | 91.7 | 110 | 95.5 | 88.8 | 94.5 | 85.0 |
| | Pool B | 97.6 | 99.6 | 87.2 | 86.4 | 106 | 88.4 | 82.2 | 88.7 | 78.8 |
| **Mean** | | **105** | **106** | **94.4** | **89.2** | **112** | **94.1** | **87.4** | **92.8** | **84.5** |
| **%RSD** | | **6%** | **4%** | **5%** | **5%** | **5%** | **5%** | **6%** | **5%** | **6%** |
| | | | | | | | | | | |
| | | **PNT** | **TPA** | **VPA** | **CZP** | **DIA** | **DM-CLBZ** | **NTZ** | **NDIA** | **CLBZ** |
| **QC LOQ** | Pool A | 78.0 | 75.9 | 81.1 | 105 | 80.3 | 77.3 | 82.7 | 84.2 | 84.4 |
| | Pool B | 85.8 | 86.8 | 87.3 | 125 | 96.1 | 84.2 | 93.9 | 121* | 93.5 |
| **QC A** | Pool A | 82.1 | 77.5 | 83.1 | 83.8 | 77.1 | 83.2 | 85.0 | 85.4 | 84.0 |
| | Pool B | 80.0 | 79.4 | 85.0 | 94.9 | 82.4 | 88.1 | 86.0 | 94.0 | 84.9 |
| **QC B** | Pool A | 89.6 | 88.1 | 93.6 | 92.3 | 88.0 | 95.5 | 92.9 | 93.0 | 95.0 |
| | Pool B | 89.2 | 86.9 | 91.7 | 89.1 | 89.0 | 93.7 | 91.3 | 97.2 | 94.1 |
| **QC C** | Pool A | 83.8 | 79.3 | 88.0 | 89.2 | 86.0 | 90.2 | 89.2 | 90.1 | 89.0 |
| | Pool B | 77.2 | 73.7 | 80.4 | 84.3 | 79.1 | 83.2 | 82.5 | 84.4 | 81.5 |
| **Mean** | | 83.2 | 81.0 | 86.3 | 91.2 | 84.8 | 86.9 | 87.9 | 89.8 | 88.3 |
| **%RSD** | | 6% | 7% | 6% | 8% | 7% | 7% | 5% | 6% | 6% |

*(*) Excluded values due to plasma contamination.*

## 6. Conclusion

LC-MS/MS has unique advantages and capabilities which make this technique a key technology asset in TDM laboratories. Advances in hardware engineering and software design are making a high impact for laboratories without the need for highly trained operators and this development is likely to continue to further automate assays and to expand the number of drugs measured by TDM. In this brief review, automated solutions for immunosuppressants and anticonvulsants have highlighted the considerable advance in integrated system designs for routine clinical pathology laboratories using LC-MS/MS.

*Table 8: Anticonvulsant QC Results*

| | | **CBZ** | **EPO-CBZ** | **GBP** | **LMT** | **LVT** | **PGB** | **ZNA** | **LCA** | **PBR** |
|---|---|---|---|---|---|---|---|---|---|---|
| **QC LOQ** | Mean Accuracy | 103 | 99.6 | 110 | 105 | 100 | 110 | 115 | 100 | 109 |
| | %RSD | 2% | 2% | 0.8% | 0.5% | 3% | 3% | 0.7% | 1% | 8% |
| **QC A** | Mean Accuracy | 103 | 98.1 | 102 | 99.6 | 98.1 | 108 | 94.4 | 106 | 97.2 |
| | %RSD | 3% | 3% | 0.5% | 0.8% | 3% | 0.5% | 0.3% | 1% | 5% |
| **QC B** | Mean Accuracy | 100 | 103 | 98.9 | 98.1 | 100 | 98.1 | 95.1 | 100 | 99.1 |
| | %RSD | 3% | 2% | 0.2% | 0.9% | 2% | 1% | 0.8% | 1% | 3% |
| **QC C** | Mean Accuracy | 110 | 111 | 103 | 103 | 107 | 103 | 104 | 102 | 98.5 |
| | %RSD | 3% | 4% | 0.3% | 1% | 3% | 0.3% | 0.6% | 1% | 1% |
| | | **PNT** | **TPA** | **VPA** | **CZP** | **DIA** | **DM-CLBZ** | **NTZ** | **NDIA** | **CLBZ** |
| **QC LOQ** | Mean Accuracy | 105 | 111 | 102 | 112 | 90.3 | 102 | 102 | 89.5 | 107 |
| | %RSD | 12% | 3% | 3% | 16% | 2% | 4% | 6% | 3% | 2% |
| **QC A** | Mean Accuracy | 104 | 98.5 | 97.0 | 93.3 | 94.9 | 100 | 94.2 | 93.3 | 97.7 |
| | %RSD | 4% | 2% | 2% | 11% | 0.6% | 2% | 2% | 1% | 2% |
| **QC B** | Mean Accuracy | 100 | 96.1 | 98.0 | 95.7 | 93.4 | 97.6 | 98.3 | 92.0 | 97.7 |
| | %RSD | 3% | 2% | 1% | 7% | 0.7% | 1% | 1% | 0.7% | 0.7% |
| **QC C** | Mean Accuracy | 98.6 | 99.7 | 100 | 102 | 104 | 103 | 104 | 102 | 107 |
| | %RSD | 3% | 2% | 2% | 3% | 2% | 1% | 0.5% | 2% | 2% |

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