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Application News

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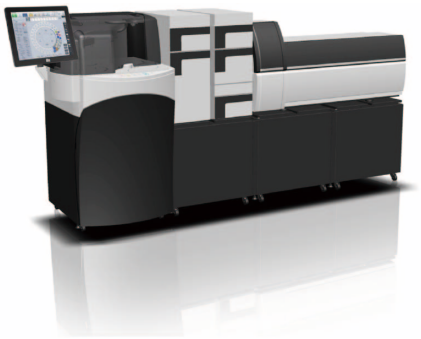
Liquid Chromatography Mass Spectrometry

# High-Throughput Optimization of Therapeutic Drug Monitoring Using Fully Automated Sample Preparation LC/MS/MS System (CLAM-2000 + LCMS-8040)

Therapeutic drug monitoring (TDM) is a series of processes where the blood concentration of drugs in a patient is measured to determine the optimal dose and method of administration for an individual based on pharmacokinetic and pharmacodynamic analysis. TDM is used during drug treatment with drugs that pose administration management difficulties, such as drugs with a narrow therapeutic range or with an effective range and toxic range that are close to each other. High performance liquid chromatography (HPLC) has been the main analytical method used with TDM, but recently liquid chromatography-mass spectrometry (LC/MS/MS) is being used to improve analytical accuracy and precision based on its superior selectivity.
LC/MS/MS normally requires sample preparation steps such as deproteinization and dilution to analyze a blood serum or blood plasma sample. These steps introduce the risk of error or variability occurring based on operator skill. The volume of work performed by an operator also increases in accordance with the number of samples. Therefore, the sample preparation process can become the bottleneck of an analytical workflow when analyzing a large number of samples.

This article introduces the results of TDM using a fully automated sample preparation LC/MS/MS system comprised of the CLAM-2000 fully automated LCMS sample preparation unit and the LCMS-8040 high performance liquid chromatograph-mass spectrometer. This system resolves the above-mentioned problems associated with TDM, and achieves TDM research results on a fast and high-precision analytical workflow.



**Fully Automated Sample Preparation LC/MS/MS System**

## High-Throughput Analytical Workflow for Antiepileptic Drug Analysis

We introduce an example simultaneous analysis of seven antiepileptic drugs and drug active metabolites in blood serum using a fully automated sample preparation LC/MS/MS system.
Preparation of blood serum samples for analysis normally requires deproteinization by the addition of organic solvent, and then centrifugal separation of solid components followed by supernatant recovery. The fully automated sample preparation LC/MS/MS system only requires placing of the blood collection tube in the system, as the system performs all these preparation steps automatically, followed by LC/MS/MS analysis (Fig. 1).
Preparation of the next sample can also be performed in parallel with LC/MS/MS analysis, which can greatly reduce the time required for each sample analysis. In our example, a per-sample cycle time including analysis of 9 minutes is achieved.

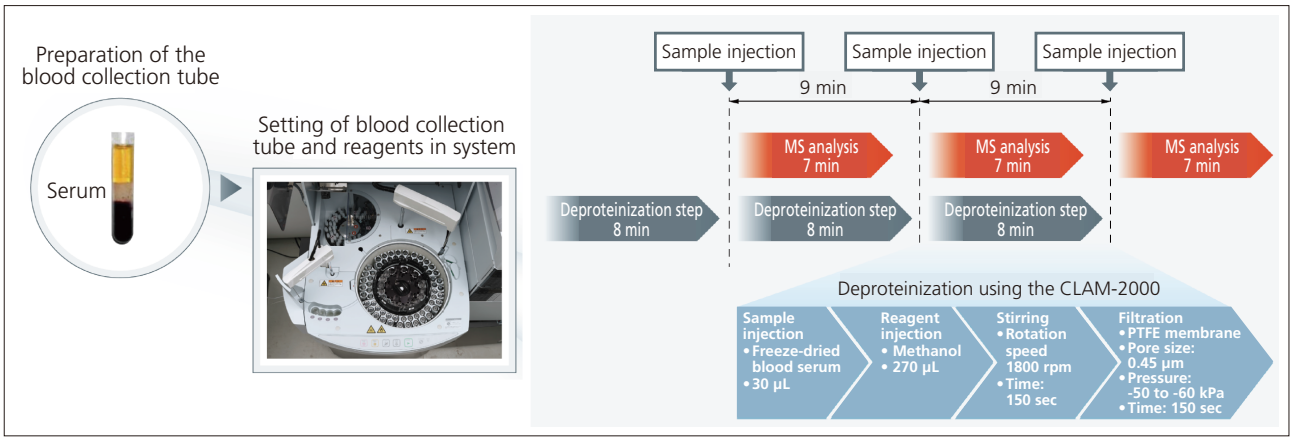



**Fig. 1 Workflow for Simultaneous Analysis of Antiepileptic Drugs in Blood Serum Using Fully Automated Sample Preparation LC/MS/MS System**

Fig. 2 shows the mass chromatogram for a control sample consisting of seven antiepileptic drugs and drug metabolites added to human blood serum. Because LC/MS/MS can detect target drugs selectively based on the mass and structure of those drugs, the results show no apparent interference from other constituents in the blood serum.

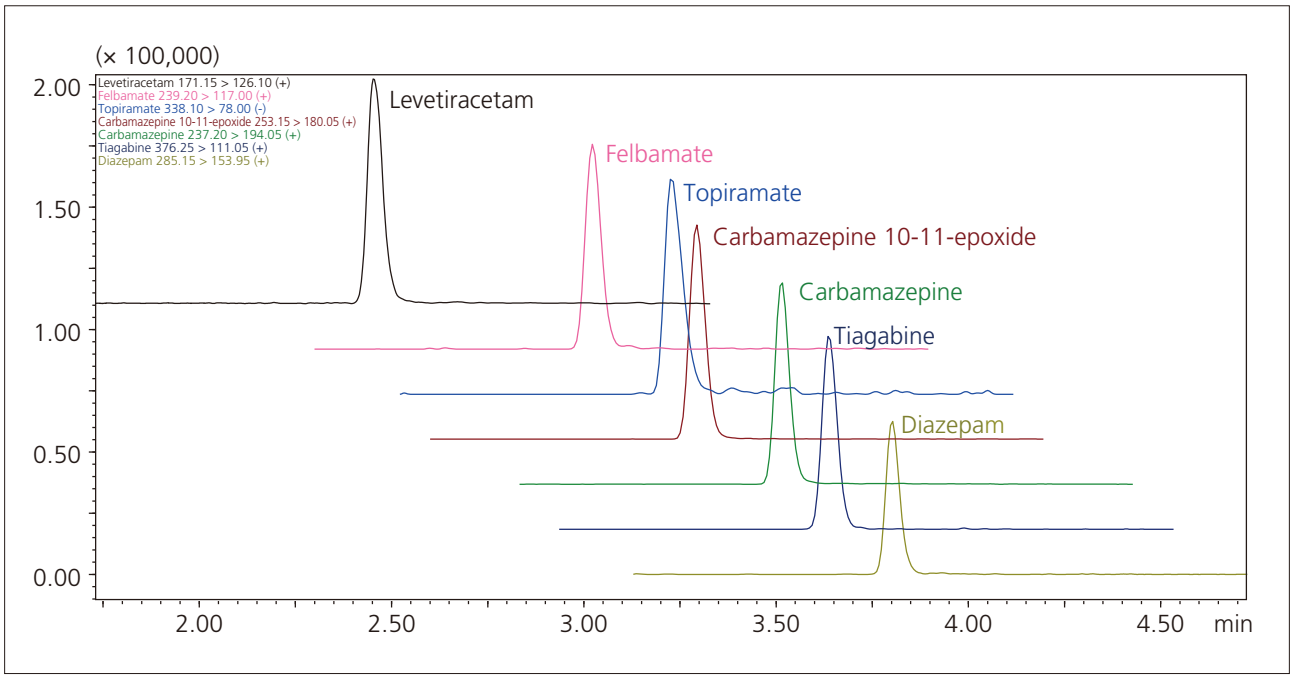



**Fig. 2 Mass Chromatogram of Seven Antiepileptic Drugs and Drug Metabolites in a Control Serum Sample**

Calibration curves were prepared by continuous analysis with fully automated sample preparation and analysis, and used to assess accuracy and precision (repeatability). Good linearity was obtained across the set calibration curve range for each antiepileptic drug (Fig. 3), with accuracy within 100 % ±15 % over the entire measurement range including the minimum limit of quantification. Similarly, precision was measured at a %RSD of within 15 %, showing that good repeatability was achieved (Table 1).

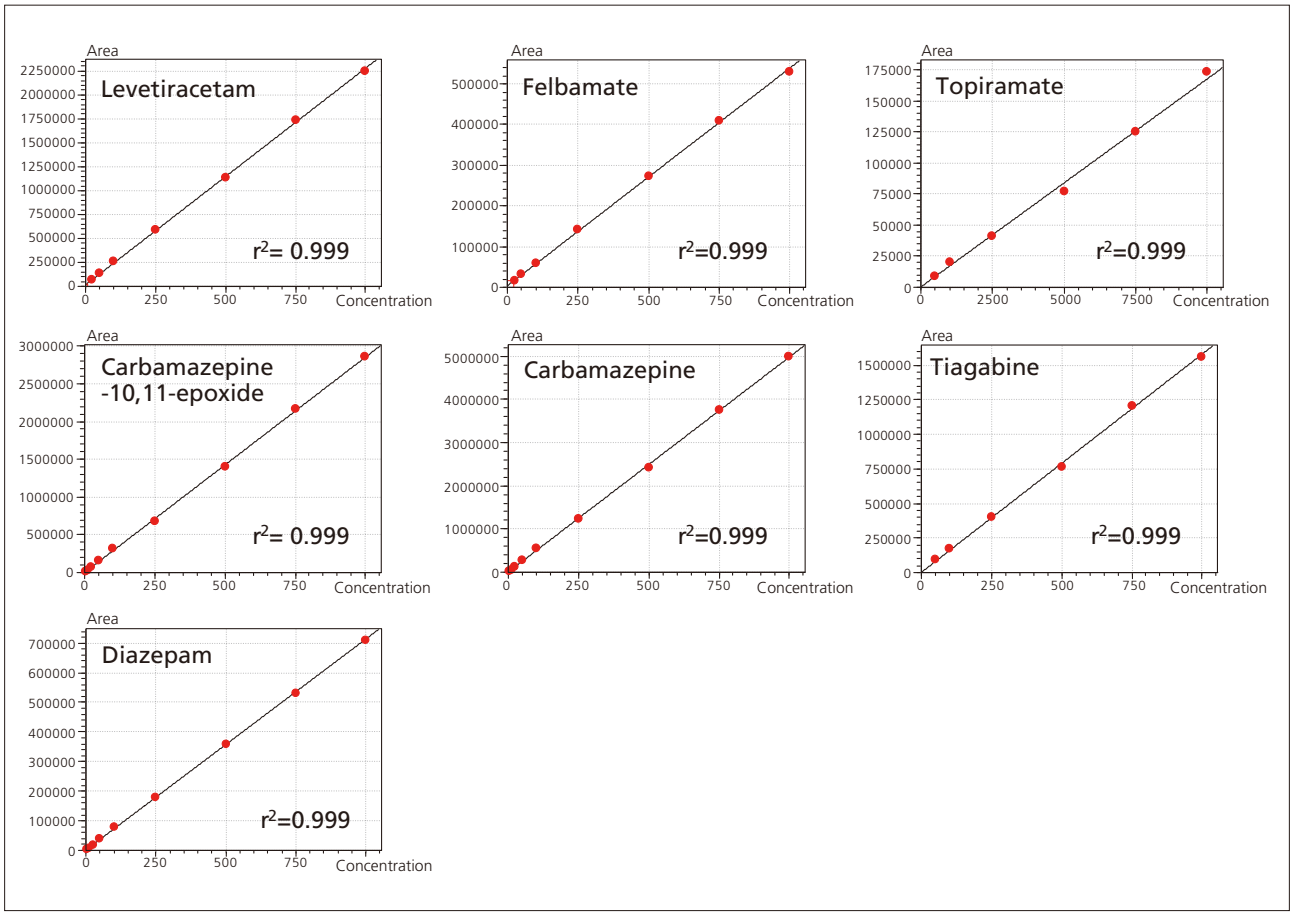



**Fig. 3 Calibration Curves of Seven Antiepileptic Drugs and Drug Metabolites**

**Table 1 Results of Validation Test for Simultaneous Analysis of Antiepileptic Drugs**

| Compounds | Range (ng/mL) | QC samples concentration (ng/mL) | | | Accuracy (%) | | | % RSD (n=6) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | LLOQ | Medium | ULOQ | LLOQ | Medium | ULOQ | LLOQ | Medium | ULOQ |
| Levetiracetam | 10 - 750 | 10 | 100 | 750 | 94.6 | 106.1 | 99.2 | 3.42 | 1.23 | 1.98 |
| Felbamate | 25 - 1000 | 25 | 250 | 1000 | 98.6 | 101.8 | 99.6 | 6.28 | 1.88 | 1.50 |
| Topiramate | 500 - 10000 | 500 | 2500 | 10000 | 102.3 | 97.1 | 100.6 | 6.71 | 3.58 | 2.96 |
| Carbamazepine-10, 11-epoxide | 5 - 1000 | 5 | 100 | 1000 | 92.9 | 107.8 | 99.3 | 7.48 | 3.32 | 1.41 |
| Carbamazepine | 10 - 1000 | 10 | 100 | 1000 | 90.6 | 110.3 | 99.1 | 3.79 | 3.42 | 1.19 |
| Tiagabine | 50 - 1000 | 50 | 250 | 1000 | 98.5 | 101.9 | 99.6 | 1.95 | 2.00 | 1.26 |
| Diazepam | 5 - 1000 | 5 | 250 | 1000 | 98.1 | 102.4 | 99.5 | 4.61 | 1.50 | 1.53 |

**Table 2 Analytical Conditions for Antiepileptic Drugs**

| | |
|---|---|
| Column | : Inertsil ODS-4 (50 mm L. × 2.1 mm I.D., 2 µm) |
| Mobile Phase | : A 10 mmol/L Ammonium acetate - Water |
| | : B Methanol |
| Flowrate | : 0.4 mL/min |
| Time Program | : B. Conc. 3 % (0 - 0.5 min) - 90 % (3.0 - 5.0 min) - 3 % (5.01 - 7.0 min) |
| Column Temperature | : 40 °C |
| Injection Volume | : 1 µL |
| Probe Voltage | : 4.5 kV / - 3.5 kV (ESI-positive / negative mode) |
| DL Temperature | : 150 °C |
| Block Heater Temperature | : 400 °C |
| Nebulizing Gas Flow | : 3 L/min |
| Drying Gas Flow | : 10 L/min |
| MRM Transition | : Levetiracetam (+) *m/z* 171.15 > 126.10, Felbamate (+) *m/z* 239.20 > 117.00, Carbamazepine-10,11-epoxide (+) *m/z* 253.15 > 180.05, Carbamazepine (+) *m/z* 237.20 > 194.05, Tiagabine (+) *m/z* 376.25 > 111.05, Diazepam (+) *m/z* 285.15 > 153.95, Topiramate (-) *m/z* 338.10 > 78.00 |

## System Validation for Antiarrhythmic Drugs Analysis

TMD is used with a wide variety of drugs, and the physicochemical properties of these drugs differ individually. Therefore, confirming whether a given series of standard operations, which includes the process steps, tools, instruments and equipment used in an analytical workflow, are appropriate for the target drug is important for ensuring the analytical results obtained are valid. We introduce an example validation of sample preparation and analysis operations using antiarrhythmic drugs with very different physicochemical properties, and in particular very different hydrophilic properties.

We chose the highly hydrophilic drug sotalol (partition coefficient: log P=2.6342) and the highly hydrophobic drug amiodarone (log P=6.9326) and its active metabolite N-desethylamiodarone were chosen, and performed simultaneous analysis using the fully automated sample preparation LC/MS/MS system (Fig. 4).

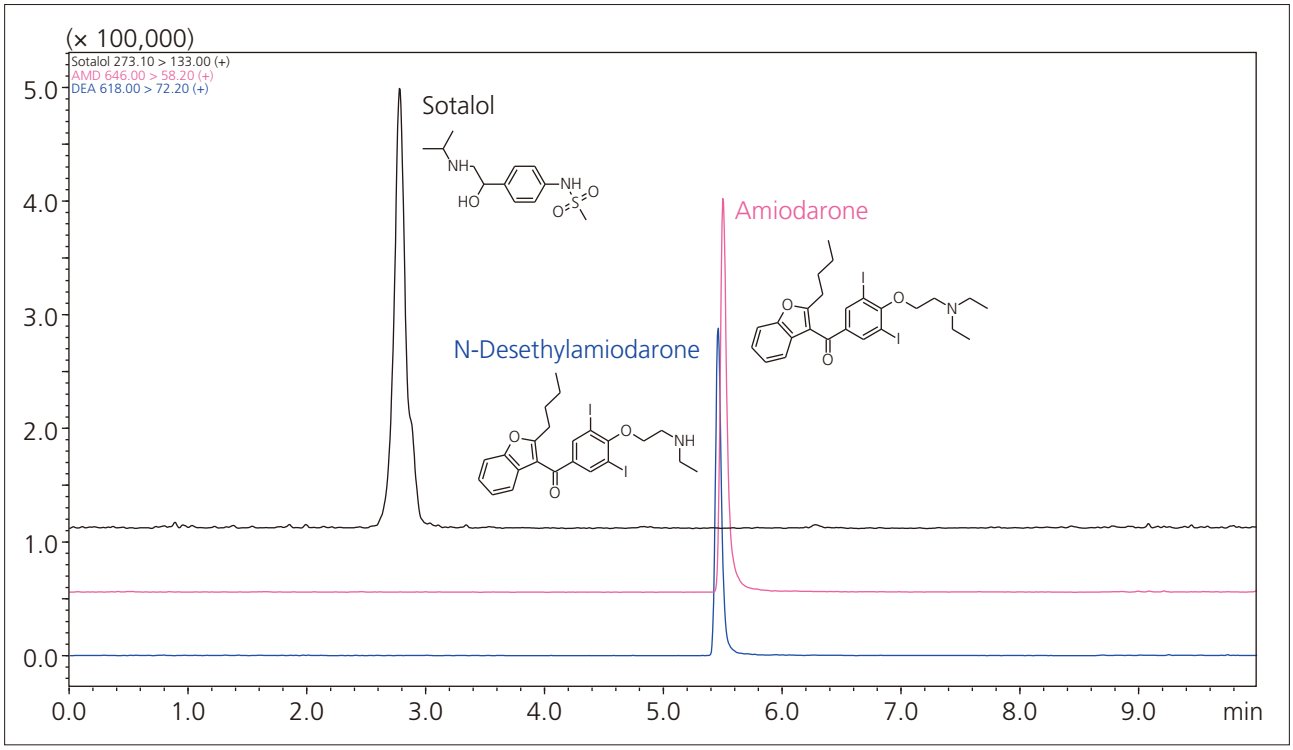



**Fig. 4 Mass Chromatogram of Three Antiarrhythmic Drugs and Drug Metabolite in a Control Serum Sample**

Calibration curves were prepared by continuous analysis, then used to validate accuracy and precision (repeatability). Good linearity was obtained across the set calibration curve range for each of the highly hydrophilic drug sotalol and the highly hydrophobic drug amiodarone and its active metabolite N-desethylamiodarone, with accuracy within 100 % ±15 % over the entire measurement range including the minimum limit of quantification. Similarly, precision was measured at a %RSD of within 15 %, showing that good repeatability was achieved (Table 3). These results indicate that sample preparation and analysis performed using the fully automated sample preparation system is suitable for a wide range of hydrophilic and hydrophobic drugs.

**Table 3 Results of Validation Test for Simultaneous Analysis of Antiarrhythmic Drugs**

| Compounds | Range (ng/mL) | QC samples concentration (ng/mL) | | | Accuracy (%) | | | % RSD (n=6) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | LLOQ | Medium | High | LLOQ | Medium | High | LLOQ | Medium | High |
| Sotalol | 100-5000 | 100 | 1000 | 2000 | 107.0 | 101.2 | 101.1 | 3.20 | 1.83 | 1.80 |
| Amiodarone | 100-5000 | 100 | 1000 | 2000 | 99.2 | 102.6 | 100.6 | 3.78 | 1.66 | 1.99 |
| N-Desethylamiodarone | 100-5000 | 100 | 1000 | 2000 | 101.2 | 103.3 | 100.1 | 4.22 | 1.48 | 3.01 |

**Table 4 Preparation Conditions for Antiarrhythmic Drugs**

| | |
|---|---|
| Sample Volume | : 50 µL |
| Reagent | : Acetonitrile 200 µL |
| Shaking | : 90 sec, 1900 rpm |
| Filtration | : 150 sec |

**Table 5 Analytical Conditions for Antiarrhythmic Drugs**

| | |
|---|---|
| Column | : Mastro C18 (100 mm L. × 2.1 mm I.D., 3 µm) |
| Mobile Phase | : A 0.1 % Formic acid - Water |
| | : B 0.1 % Formic acid - Methanol |
| Flowrate | : 0.4 mL/min |
| Time Program | : B. Conc. 5 % (0 - 1.5 min) - 100 % (5.5 - 7.5 min) - 5 % (7.51 - 10 min) |
| Column Temperature | : 40 °C |
| Injection Volume | : 0.3 µL |
| Probe Voltage | : 4.5 kV (ESI-positive mode) |
| DL Temperature | : 250 °C |
| Block Heater Temperature | : 400 °C |
| Nebulizing Gas Flow | : 3 L/min |
| Drying Gas Flow | : 15 L/min |
| MRM Transition | : Sotalol (+) *m/z* 273.1 > 133.0, Amiodarone (+) *m/z* 646.0 > 58.2, N-Desethylamiodarone (+) *m/z* 618.0 > 72.2 |

## Conclusion

Results indicate that the fully automated sample preparation LC/MS/MS system can eliminate the risk of error or variability introduced by manual sample preparation that has been a problem for TDM, and also indicate this system can implement a quick and high-precision analytical workflow that is compatible with drugs with a wide variety of physicochemical properties. We anticipate the fully automated sample preparation LC/MS/MS system will contribute to improved analytical reliability and throughput in TDM.

<Acknowledgments>
This research was performed with considerable help from Dr. Takeshi Kuwahara of the Pharmacy Department, National Cerebral and Cardiovascular Center in Japan.

[References]

1) Guidance for Industry: Bioanalytical Method Validation (2001, US FDA)
2) Guideline on Bioanalytical Method Validation in Pharmaceutical Development (Japan's MHLW, 2013)

Notes

- The products mentioned in this article have not been approved/certified as medical devices according to the Pharmaceutical and Medical Device Act in Japan.
- The analytical methods mentioned in this article cannot be used for diagnostic purposes.

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