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## 1. Introduction

Simplifications in LCMS instrumentation have made MS a viable option for clinical research. This technique has the advantage of specificity, accuracy, and reduced reagent costs compared to immunoassays. The ability to support various analysis methods on a single system is a key feature. However, it is not always easy to quickly alternate between several different analytical methods. Also, automation is an essential function in aiming for a better quality of results and better comfort for the users in testing laboratories. However, lately, the automation of biological sample extraction, directly coupled to LCMS, has proven to be a challenge in the field.

A collaboration between the University Medical Center Göttingen (UMG) and Shimadzu Corporation was built to jointly develop and validate multiple analytical methods for therapeutic drugs, using a fully automated platform (Figure 1.). The purpose is the development and the validation of a unified methods set for LCMS, for 24/7 therapeutic drug analysis, with a single system configuration. It means that methods can alternate easily with no need for human intervention, enabling smooth use in the clinical research laboratory.

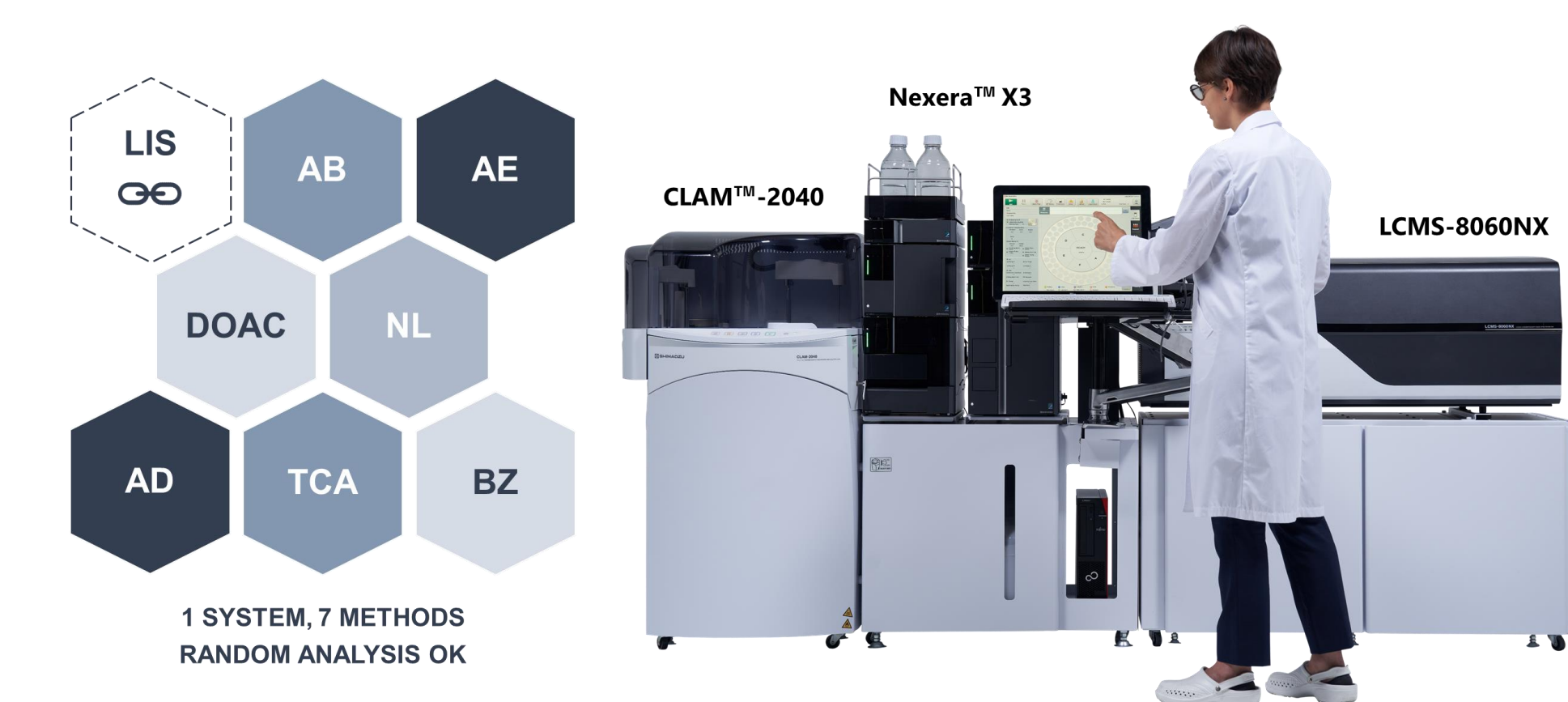


Figure 1. Fully automated platform for TDM. Overview of methods and analytical system.

## 2. Material and Methods

The evaluated analytical system was a fully automated platform, from Shimadzu Corporation, composed of a CLAM™-2030 automation module (now updated to CLAM™-2040), coupled to Nexera™ X3 UHPLC and LCMS-8060NX MS/MS. HL-7 interface standards were used for bidirectional communication between the laboratory information system (LIS) (Dedalus, Germany) and the CLAM-LC/MS/MS. The target applications were Antibiotics (AB, 16 compounds), Direct Oral

Anticoagulants (DOAC, 9 compounds), Antiepileptics (AE, 26 compounds), Neuroleptics (NL, 28 compounds), SSRI Antidepressant drugs (AD, 36 compounds), Tricyclic Antidepressants (TCA, 13 compounds), and Benzodiazepines (BZ, 35 compounds). For each class of compounds, one individual method was developed, optimized, and validated (7 methods). All methods use similar analytical conditions (Figure 2.), so that there is no need for system equilibration between two different methods. The fitness for purpose of this platform for 24/7 use was then evaluated by repeatedly requesting measurements for all methods in a random alternance.

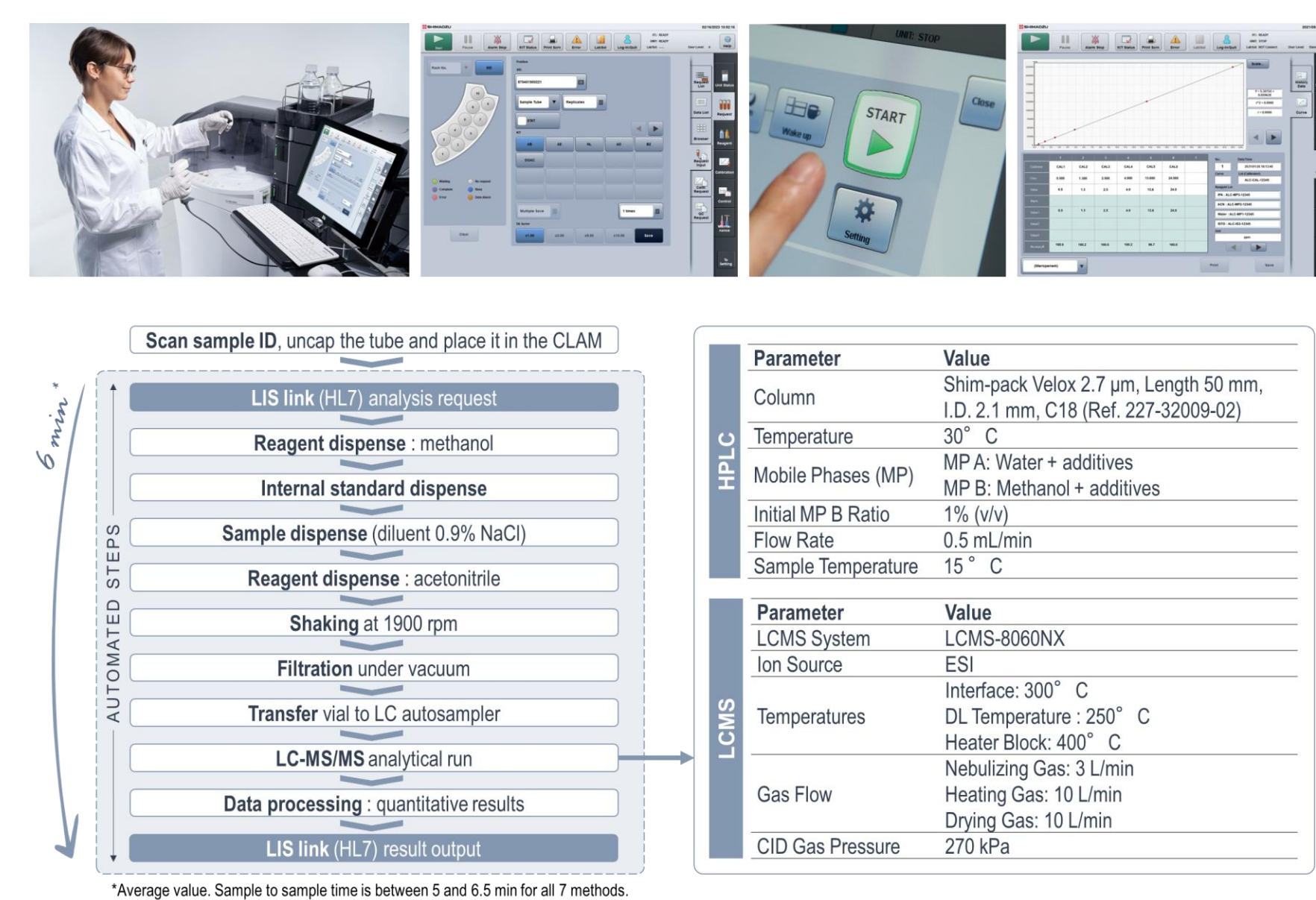


Figure 2. Unified analytical parameters (identical parameters between all 7 methods).

## 3. Results

At the time of preparing this poster the antibiotic method is still under validation at UMG hospital. All other methods validations have already been completed. Validation criteria are detailed hereafter. Individual methods validations include isobars resolution (resolution above 1), calibration accuracy (QC and calibrant accuracy within 80-120%), LCMS repeatability (area, area ratio and ion ratio CV below 10%), CLAM-LC/MS/MS method repeatability (area, area ratio and ion ratio CV below 15%), day-to-day intermediate precision (area, area ratio and ion ratio CV below 15%), mobile phase stability (retention time deviation below 2% after 2 weeks), LLOQ confirmation (signal-to-noise ratio above 10 and area CV below 15%), matrix effect evaluation (matrix factor within 50-120%), absence of carryover confirmation (blank to LLOQ area ratio below 20%) and ring trial analysis (sample concentration accuracy within

80-120%). Also, repeated measurements for all methods in random alternance (only partially completed) are promising and are showing results within the acceptance criteria (accuracy within 80-120% and CV below 15%). A selection of the validation results is shown hereafter. In Figure 3., for each method, results for 6 representative compounds are presented: the calibration accuracies (QC and calibrant), the CLAM-LC/MS/MS method repeatability (QC area ratio CV, intra-day), and the day-to-day intermediate precision (QC area ratio CV, inter-day). All passed the validation criteria. Figure 4. is showing example ring trials analysis results for Antidepressant drugs and Benzodiazepines.

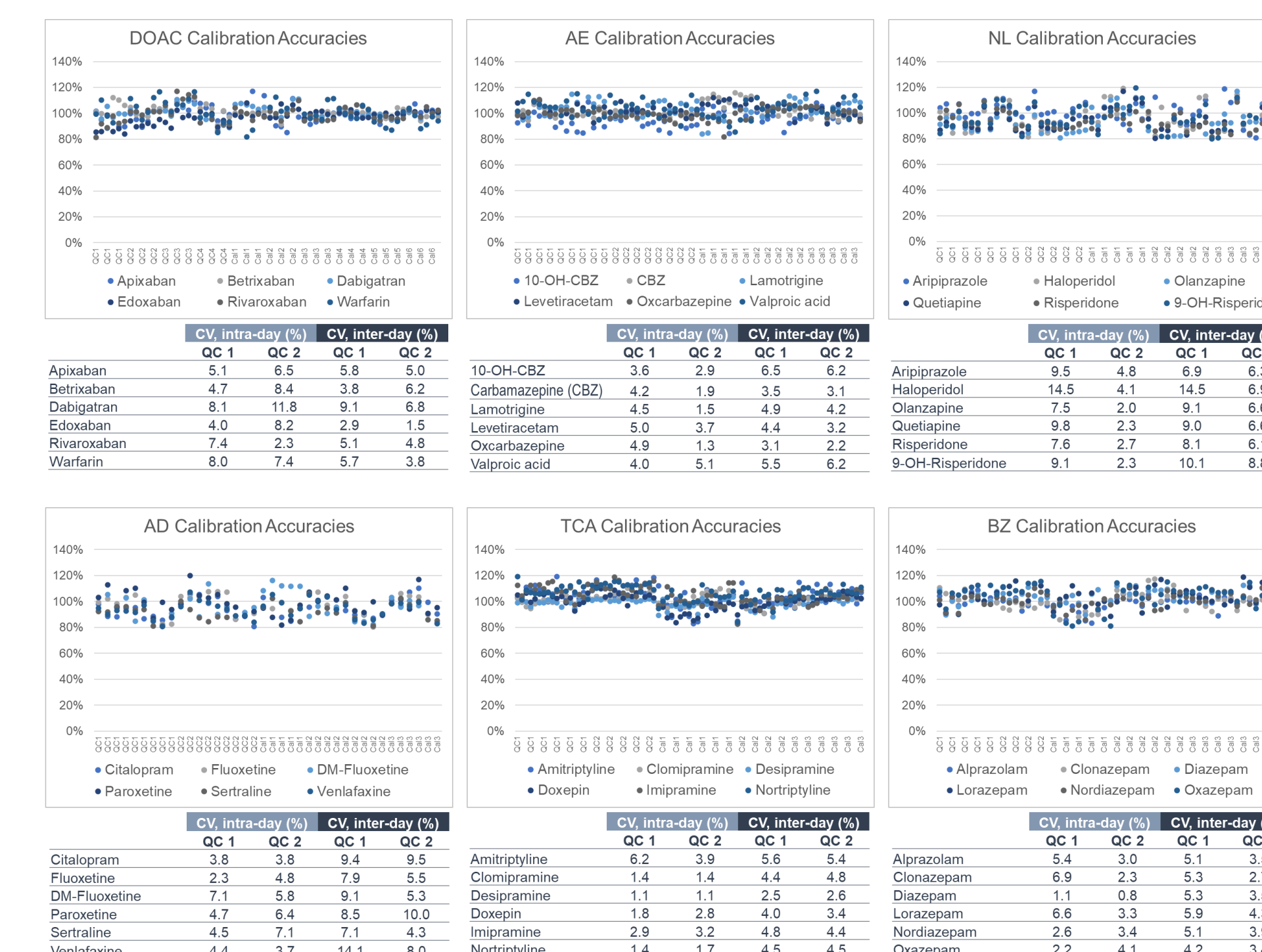


Figure 3. Calibration accuracies and QC area ratio CV, intra-day and inter-day. For each method, 6 representative compounds are presented.

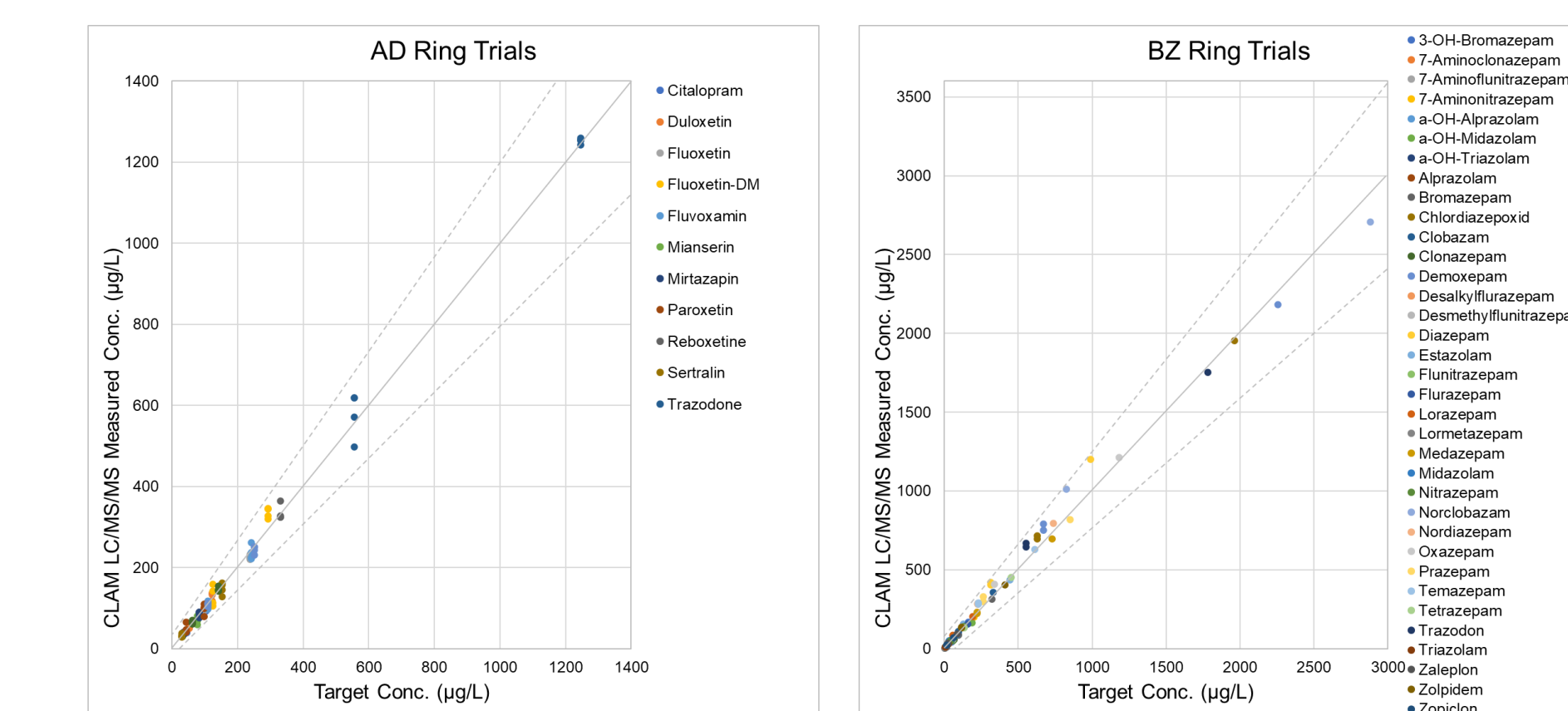


Figure 4. Ring trials analysis results for Antidepressants (AD) and Benzodiazepines (BZ).

## 4. Discussion

This methodology has demonstrated to have various advantages over typical conventional LCMS methods (Figure 5.). These include (but are not limited to): the calibration stability (daily calibration is not necessary), the ease of access of the system which requires low technical knowledge, the comfort of the automated sample preparation enabling less than 10 min time between receiving the sample and the result output, the easy switch between different analytical methods with no need for purge or equilibration, the possibility to analyze samples in a random order of arrival (including requests for different methods), and the LIS connectivity (HL7 interface).

Feature	Typical LCMS Method Manual sample prep. + LCMS	Novel Method CLAM-LC/MS/MS
Calibration stability	Daily calibration	More than 1 week
Technical knowledge required	High	Low
Automated online sample preparation	Not available	Yes
Time from sample to result	More than 30 min	Less than 10 min
Procedure between methods	Purge, System equilibration	No need > smooth switch
Random order analysis (various methods)	Not available	Available
LIS link (HL7 interface)	Indirect	Yes
Suitable for 24/7	Difficult	Yes

Figure 5. Novel method advantages over typical conventional LCMS method.

## 5. Conclusion

“Convincing” “Easy handling” “Conceivable for 24/7”

This strategy proved its fitness for purpose for 24/7 therapeutic drug analysis by LCMS. The fast LCMS methods which can alternate smoothly, and the automated sample extraction enable robust therapeutic drug analysis with a high throughput and at low cost, while improving the user comfort.

**Disclaimer: The products and applications in this presentation are intended for Research Use Only (RUO). Not for use in diagnostic procedures.**

## COI Disclosure Information

This study was funded by Shimadzu Corporation.