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Development and Implementation of a Practical Training and Assessment System for LC–MS/MS Sample Preparation in Clinical Laboratory Medicine: A feasibility Study

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Abstract: Background: Liquid chromatography–tandem mass spectrometry (LC–MS/MS) is indispensable in clinical laboratories, yet standardized and effective training programs for short-term rotating trainees in clinical laboratory medicine remain underdeveloped.
Objectives: To develop and evaluate a standardized competency-based on-the-job training (OJT) system for LC–MS/MS sample preparation, incorporating quantitative performance metrics to objectively assess trainee competency.
Methods: This study enrolled 43 participants, including 11 undergraduates, 13 postgraduates, and 19 continuing medical education (CME) specialists. Participants completed pipetting proficiency test and hands-on training for voriconazole and antipsychotic drug sample preparation. Competency was assessed using intraindividual variation (IIV) and bias derived from internal standard (IS) response data, with predefined thresholds. Troubleshooting discussion and feedback survey were conducted immediately after the training.
Results: Trainees exhibited superior pipetting accuracy and precision for water versus organic solvents (dichloromethane). Postgraduates outperformed undergraduates and CME specialists in terms of the precision of sample preparation, particularly for antipsychotic drugs. The training system achieved high satisfaction rates: 74.4% reported increased interest in LC–MS/MS technology, 97.7% acknowledged improved hands-on skills, and 86.1% perceived no added rotation burden. Further troubleshooting revealed that organic solvent handling, supernatant transfer consistency and prior experience were the main factors affecting the trainees' LC–MS/MS sample preparation performance.
Conclusion: This study establishes the first OJT system for LC-MS/MS that links quantitative metrics to hands-on competency, addressing a critical practical training gap for short-term rotations in clinical laboratory medicine. The framework's adaptability and trainee-centric design offer a scalable model for standardizing skill assessment in clinical laboratories, with potential applications to other complex techniques.

Keywords: Competence, on-the-job training, LC–MS/MS, pipetting proficiency

1. Introduction

In recent years, liquid chromatography–tandem mass spectrometry (LC–MS/MS) has become increasingly important in the diagnostic niches of laboratory medicine and is recognized as unreplaceable by alternative technologies[1]. Owing to its exceptional specificity, sensitivity, and multiplexing capabilities, this advanced technique has been widely applied in various clinical laboratory settings, including therapeutic drug monitoring (TDM), toxicology, endocrinology, and newborn screening[2]. The significant contributions of LC–MS/MS to biomarker discovery and personalized medicine have further solidified its vital position in

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modern laboratory medicine. Recognizing the growing importance of LC–MS/MS, the Chinese Journal of Laboratory Medicine dedicated a special issue to mass spectrometry technology eight years ago, highlighting its vital role in the field of laboratory medicine[3]. However, in the context of modern medicine, the implementation of LC–MS/MS in routine clinical laboratories still faces several challenges. The large capital expenses for instrument purchases, standardization of assays across the board, and requirements for highly qualified technical staff remain the main issues to be addressed[4, 5]. In particular, compared with the development wave of "clinical mass spectrum technology", expertise in clinical LC–MS/MS is extremely limited. This disparity underscores the critical need for comprehensive educational initiatives to increase the expertise of laboratory personnel in LC–MS/MS applications.

In contrast to matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, which is widely applied in microbiology laboratories supported by well-integrated training programs at all levels, the development of systematic educational frameworks for clinical LC–MS/MS remains relatively limited. Over the past decade, a series of educational initiatives have been developed to enhance the general understanding of LC–MS/MS technology, with numerous activities focused on enhancing technical competencies in method development, validation, and clinical applications[6]. Major professional organizations have played a pivotal role in advancing LC–MS/MS education through diverse platforms. Notably, the Japanese Society for Biomedical Mass Spectrometry (JSBMS), the German Society for Clinical Chemistry and Laboratory Medicine (DGKL), and the International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT) have contributed substantially through the development of educational resources, including webinars, tutorial sessions, instructional videos, and specialized publications[7, 8]. However, a gap persists in the establishment of standardized training curricula and competency assessment systems for personnel participating short-term rotations in the clinical mass spectrometry department.

Currently, LC–MS/MS suffers from low automation capabilities and relies on multistep manual sample preparation workflows. Effective sample preprocessing, which is critical for achieving optimal analyte recovery and eliminating matrix interference, requires specialized hands-on experience to ensure both analytical quality and operational efficiency[9, 10]. This necessity underscores the importance of comprehensive training programs that focus on the development of practical skills in sample preparation techniques. In addition, competency assessment, which is used to confirm that training is effective and that personnel are capable of following established procedures to perform testing correctly, is also important during the education and training process[11]. In our department, the rotation time of trainees varies from 1 week to 4 weeks, and trainees often report that they are unable to conduct onsite LC–MS/MS operations owing to the complexity of procedures and time constraints, which discourages them from learning practical LC–MS/MS skills. To address these problems, the development of standardized on-the-job training (OJT) programs, coupled with robust testing and competency evaluation systems, is essential for optimizing the effectiveness of short-term rotations in LC–MS/MS practice. Such structured training frameworks would not only enhance skill acquisition but also improve the overall quality and consistency of LC–MS/MS operations in clinical laboratories.

In this study, we therefore aimed to develop an effective training and evaluation system for LC–MS/MS sample preparation that is specifically tailored for rotating trainees in clinical laboratory medicine departments. Furthermore, we conducted a preliminary comparative analysis of training outcomes across different trainee cohorts, including undergraduate interns, postgraduate students, and continuing medical education (CME) specialists, to evaluate the effectiveness of the system across various levels of professional experience.

2. Materials and methods

2.1 Study design and participants

This single-center, observational study aimed to explore the feasibility of using the "internal standard (IS) method" as a competency assessment tool for evaluating trainee performance in LC–MS/MS sample preprocessing. Undergraduate interns, postgraduate students and CME specialists who rotated in the 'therapeutic drug monitoring (TDM)' group of the Department of Laboratory Medicine at West China Hospital of Sichuan University were enrolled in the study. First, all the participants learned the standard operation procedures (SOPs) of the chosen items both on their own and through onsite teaching prior to manual operation. Pipetting proficiency tests were conducted to obtain baseline information on the participants' pipetting accuracy and precision. Then, participants completed the sample preparation independently, and the IS response was determined with the help of supervisors. Finally, on the basis of the performance of each participant, operation-related problems were identified after discussion. Trainee satisfaction with this OJT approach was assessed with a feedback questionnaire. The flowchart of the study is summarized in **Figure 1**.

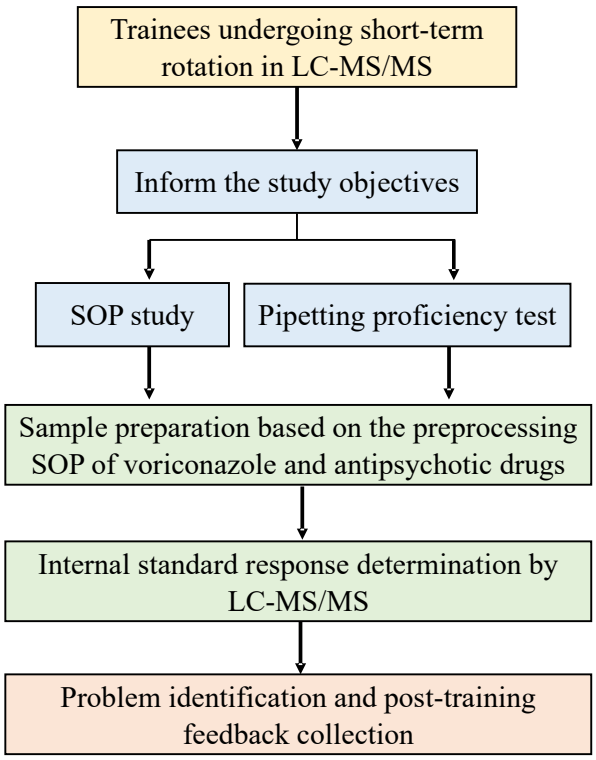

Figure 1 Study flowchart

2.2 Pipetting proficiency test before operation on LC-MS/MS samples

A gravimetric method was applied prior to sample preparation to characterize the baseline pipetting proficiency of the participants. Fluids, including water, methyltert-butyl ether (MTBE), and dichloromethane, with varying physical properties and different volumes (50 μl, 200 μl and 1000 μl) were used for the pipetting proficiency test. Specifically, empty 1.5 ml or 2 ml Eppendorf tubes were initially weighed, and then the tubes were reweighted with an electronic celestial scale after the corresponding volume and type of liquid was pipetted. Designated pipettors were used by all operators for pipetting, and the data were recorded on a predesigned sheet. The pipetting accuracy was determined by comparing the measured mass (derived from weight differences) with the theoretical target mass, which was calculated as the product of the pipetting volume and the respective liquid density (**Supplementary Table 1**). To ensure robust statistical analysis, each operator performed five replicates per liquid–volume combination. Intraindividual variation (IIV) quantifies pipetting precision by measuring the consistency of trainee's repeated performance. It was calculated as: IIV (%) = $\left(\frac{\text{Standard deviation (SD) of fluid mass across replicates}}{\text{Mean fluid mass}}\right) \times 100$. Bias evaluates the deviation between the target mass and the measured mass, served as the primary metric for accuracy evaluation. It was calculated as: Bias (%) = $\left(\frac{\text{Detected fluid mass} - \text{Target fluid mass}}{\text{Target fluid mass}}\right) \times 100$.

2.3 LC-MS/MS sample preparation workflow

In our center, therapeutic drug monitoring (TDM) for voriconazole and antipsychotic drugs is routinely performed as part of the standard clinical practice. They both use liquid-liquid extraction method to extract targets from samples, which is one of the most commonly used extraction methods for LC-MS/MS sample preparation. Additionally, the sample preparation for voriconazole requires precise upper-layer collection of low-density organic phase, while antipsychotic drug requires direct lower-layer harvesting of high-density solvent, which provides multidimensional technical training opportunities to improve trainees' hands-on competencies. Therefore, we choose the preprocessing of these two items as the representative training modules in this study.

For the implementation, the trainees were initially asked to learn the detailed contents of the SOPs on their own. The detailed SOPs are presented in **Supplementary Figures 1 and 2**. The supervisors then emphasized the key skills related to the operation. After theoretical learning, the trainees performed liquid–liquid extraction independently. The ISs for detecting voriconazole and antipsychotic drug concentrations were prepared in advance with cyproheptadine solutions at concentrations of 0.2 μg/ml and 50 ng/ml, respectively. Blank plasma was used to simulate clinical samples. The operation steps for voriconazole were as follows: 50 μL of IS (voriconazole), 100 μL of blank plasma and 1 ml of MTBE were added to a 1.5 ml Eppendorf tube. A total of 50 μL of pH 9.2 buffer was added to adjust the pH value of the solutions. The mixture was fully vortexed for 5 min, followed by centrifugation at 12,000 rpm for 5 min. Subsequently, 800 μl of the supernatant was transferred to a 2 ml glass screw cap vial. The samples were concentrated after volatilization of the organic solvent via a low-temperature vacuum concentrator. The concentrated extract was subsequently dissolved in 1 ml of mobile phase for LC–MS/MS detection (**Supplementary Figure 1**). The

operation steps for antipsychotic drugs were as follows: 50 μL of IS (antipsychotic drug), 200 μL of blank plasma and 3 ml of dichloromethane were added to a 10 ml round bottom glass tube. NaOH (1.0 mol/L) was added to adjust the solution to an alkaline environment. The mixture was vortexed for 5 min, followed by centrifugation at 2500 rpm for 5 min. A 2 ml lower layer of the supernatant was transferred to another glass tube for concentration. Then, 150 μl of mobile phase was added to dissolve the concentrated extract, which was finally transferred to a glass screw neck vial for LC–MS/MS detection (**Supplementary Figure 2**). Batch submission and data analyses for these LC–MS/MS samples were performed by supervisors.

2.4 Competency assessment

The IS response, determined by LC-MS/MS detection, is considered an objective, comprehensive and surrogate indicator for assessing sample preparation proficiency. Trainee competency for LC-MS/MS sample preparation was quantified using two metrics: accuracy (bias) and precision (intraindividual variation, IIV)**.** These metrics were calculated on the basis of IS response data from LC-MS/MS analysis of prepared samples. The bias quantifies accuracy by comparing measured IS response to a predefined target IS response. It was calculated as: Bias (%) = $\left(\frac{\text{Detected IS response}-\text{Target IS response}}{\text{Target IS response}}\right) \times 100$. The target IS responses (100000 for voriconazole; 200000 for antipsychotics) were derived from 25 validation runs by five expert technicians, ensuring the robustness. IIV evaluates precision by measuring the consistency of a trainee's repeated performance. It was calculated as: IIV (%) = $\left(\frac{\text{Standard deviation (SD) of IS response across replicates}}{\text{Mean IS response}}\right) \times 100$. According to the criteria of External Quality Assessment Programs in Laboratory Medicine of China, we set a bias within 1/3 total error allowance (TEA, 25% × 1/3 = 8.33%) was deemed acceptable. An IIV < 8.33% (1/3 TEA for voriconazole) and IIV <12.5 % (1/2 TEA for antipsychotic drugs) were considered acceptable. Otherwise, trainees require further improvement in LC-MS/MS sample preparation. Interindividual variation (Coefficient of variation, CV) reflects the differences in sample preparation performance between trainees of each group. It was calculated as: CV (%) = $\left(\frac{\text{Standard deviation (SD) of IS response across individuals}}{\text{Mean IS response}}\right) \times 100$.

2.5 Problem identification and feedback survey

After training, a discussion was conducted between supervisors and trainees to identify potential factors contributing to suboptimal results, facilitating targeted skill improvement and operational refinement. Then, a satisfaction survey was conducted by using the "Questionnaire Star" platform to gain informative feedback on how the trainees perceived the new training and assessment system. The survey items including 'increase interest in LC-MS/MS', 'improve hands-on skills', and 'increasing the burden to the rotation'. In parallel, we collected data on the participants' educational backgrounds and baseline pipetting performance metrics to enable a more robust analysis of training outcomes.

2.6 Statistical analysis

Data are presented as absolute numbers, means ± standard deviations or medians (interquartile ranges) according to the data type. The chi-square test or Fisher's exact test was used to compare categorical variables between groups. Student's t test or the Mann–Whitney U test was applied to compare continuous variables with normal and skewed distributions, respectively. All the data analyses were performed using SPSS software (version 23.0, SPSS, Inc., Chicago, IL, USA), and a two-tailed *P* value < 0.05 was considered to indicate statistical significance.

3. Results

3.1 Basic characteristics of all participants

The study cohort comprised 43 participants who completed the comprehensive OJT training and evaluation program, with a median age of 26 years (range: 22-34) and a female predominance (79.07%). Among the 43 participants, 11 fourth-year undergraduates, 13 postgraduates and 19 CME specialists from 12 different medical centers were included. The educational background varied from a high school education to a master's degree, with 50% of the participants holding a bachelor's degree (**Table 1**). The sex distributions among the undergraduate intern, postgraduate and CME specialist groups were not significantly different, but age significantly differed among the three groups, with the CME specialist group being the oldest (**Table 2**).

Table 1 Baseline characteristics of all the participants

	All
Number	43
Age	26 (22-34)
Sex	
Male	9 （20.93%）
Female	34 （79.07%）
Trainee type	
Undergraduate intern	11 （25.58%）
Postgraduate	13 （30.23%）
CME specialist	19 （44.19%）
Educational background	
High school	11 （25.58%）
Bachelor's degree	22 （51.16%）
Master's degree	10 （23.26%）

Table 2 Comparison of pipetting proficiency among different categories of participants

		All	Undergraduate interns	Postgraduates	CME specialists	*P*
Number		43	11	13	19	
Sex						0.732
	Male	9	2	0	5	
	Female	34	9	11	14	
Age		26 (22-34)	22 (21~22)	24 (23~26)	34 (28~36)	<0.0001
Pipetting proficiency test data						
50 μl Water	Mean (g)	0.0502 (0.0498~0.0505)	0.0501 (0.0500~0.0502)	0.0502 (0.0501~0.0505)	0.0503 (0.0496~0.0505)	0.381
	Bias (%)	0.35 (-0.46~0.90)	0.28 (-0.79~0.46)	0.48 (0.11~1.00)	0.60 (-0.72~0.98)	0.381
	IIV (%)	0.64 (0.31~1.37)	1.32 (0.40~2.14)	0.38 (0.31~0.75)	0.64 (0.31~1.08)	0.307
	CV-mean (%)	1.36	1.07	0.83	1.74	
200 μl Water	Mean (g)	0.1996 (0.1987~0.2005)	0.1994 (0.1984-0.2012)	0.2004 (0.2000-0.2009)	0.1993 (0.1980-0.2000)	0.065
	Bias (%)	-0.22 (-0.66~0.23)	-0.28 (-0.80~0.62)	0.19 (-0.22~0.45)	-0.36 (-1.03~0.01)	0.065
	IIV (%)	0.23 (0.13~0.53)	0.22 (0.15-0.96)	0.29 (0.16-0.53)	0.22 (0.12-0.39)	0.630
	CV-mean (%)	1.10	0.97	0.89	1.17	
1000 μl Water	Mean (g)	0.9896 (0.9869~0.9933)	0.9888 (0.9849~0.9901)	0.9909 (0.9880~0.9957)	0.9892 (0.9864~0.9937)	0.360
	Bias (%)	-1.04 (-1.31~-0.67)	-1.12 (-1.51~-0.99)	-0.91 (-1.20~-0.43)	-1.08 (-1.36~-0.63)	0.360
	IIV (%)	0.19 (0.15~0.53)	0.17 (0.12~0.42)	0.23 (0.15~0.49)	0.19 (0.10~0.51)	0.716
	CV-mean (%)	0.64	0.52	0.59	0.75	
50 μl MTBE	Mean (g)	0.0351 (0.0338-0.0358)	0.0338±0.0031	0.0354±0.0013	0.0347±0.0018	0.194
	Bias (%)	-5.11 (-8.65~-3.14)	-8.65±8.50	-4.28±3.62	-6.15±4.95	0.194

	IIV (%)	2.15 (1.54~3.19)	2.85 (2.08~4.53)	1.93 (1.41~3.15)	2.04 (1.53~3.12)	0.258
	CV-mean (%)	6.25	9.31	3.78	5.27	
200 µl MTBE	Mean (g)	0.1543 (0.1518~0.1582)	0.1539 (0.1501~0.1571)	0.1575 (0.1540~0.1615)	0.1540 (0.1518~0.1566)	0.235
	Bias (%)	4.26 (2.55~6.86)	4.00 (1.41~6.12)	6.45 (4.03~9.11)	4.07 (2.55~5.79)	0.235
	IIV (%)	1.42 (0.90~2.47)	1.47 (1.32~2.08)	1.15 (0.57~2.48)	1.44 (0.91~2.47)	0.586
	CV-mean (%)	3.73	3.99	4.88	2.50	
1000 µl MTBE	Mean (g)	0.7302 (0.7195~0.7425)	0.7226±0.0210	0.7370±0.0104	0.7305±0.0127	0.067
	Bias (%)	-1.33 (-2.78~0.34)	-2.36±2.84	-0.40±1.41	-1.29±1.72	0.067
	IIV (%)	0.87 (0.55~1.29)	0.98 (0.83~1.24)	0.87 (0.49~1.43)	0.72 (0.51~1.07)	0.603
	CV-mean (%)	2.10	2.91	1.42	1.74	
50 µl Dichloromethane	Mean (g)	0.0601 (0.0575~0.0629)	0.0606 (0.0583~0.0626)	0.0629 (0.0608~0.0638)	0.0589 (0.0573~0.0598)	0.023
	Bias (%)	-9.28 (-13.17~-5.06)	-8.48 (-11.97~-5.49)	-5.06 (-8.23~-3.67)	-11.20 (-13.44~-9.68)	0.023
	IIV (%)	3.04 (2.19~4.38)	2.47 (2.22~4.26)	3.15 (2.24~4.43)	3.14 (2.17~4.08)	0.850
	CV-mean (%)	6.51	6.65	6.39	6.20	
200 µl Dichloromethane	Mean (g)	0.2818 (0.2748~0.3016)	0.2914±0.0140	0.2888±0.0240	0.2820±0.0167	0.376
	Bias (%)	4.06±2.66	9.96±5.27	8.99±9.04	6.42±6.29	0.376
	IIV (%)	3.14 (2.28~6.10)	2.52 (2.01~3.57)	3.09 (1.84~4.20)	4.18 (2.70~6.38)	0.735
	CV-mean (%)	6.51	4.79	8.29	5.91	
1000 µl Dichloromethane	Mean (g)	1.3129 (1.2813~1.3649)	1.3012±0.0974	1.3450±0.0467	1.3128±0.0581	0.252
	Bias (%)	-0.91 (-3.30~-3.01)	-1.80±7.35	1.51±3.53	-0.92±4.38	0.252
	IIV (%)	1.92±1.11	2.03±1.04	1.41±0.71	2.17±1.29	0.149
	CV-mean (%)	5.16	7.48	3.47	4.42	

IIV: Intraindividual variation, calculated from 5 replicate data from each participant

3.2 Pipetting proficiency data

The pipetting proficiency of the individual participants was evaluated for various liquids prior to the implementation of LC–MS/MS sample preparation. The pipetting proficiency test data are presented in **Table 2**. In general, all trainees showed much better performance for water than for organic solutions in terms of both accuracy and precision, as evidenced by the bias and IIV values. With respect to the performance of pipetting organic solutions, the pipetting of dichloromethane resulted in greater bias and imprecision than did the pipetting of MBTE. As expected, we observed that the larger the pipetting scale was, the better the precision was for all the testing solutions. A further comparison of pipetting performance among undergraduate interns, postgraduates and CME specialists demonstrated that the pipetting proficiency (bias and IIV values) of the undergraduates, postgraduates and CME specialists were comparable except for the proficiency in pipetting 50 μl dichloromethane. Interindividual variation in each group was calculated on the basis of the accuracy data, which revealed that greater interindividual variation was observed in pipetting performance for organic solutions than for water.

3.3 Assessment of the trainees' competence in LC–MS/MS sample preparation

We used the IS response as a surrogate indicator to assess the participants' performance in sample preparation. The trainees performed extractions following standardized SOPs for both voriconazole and antipsychotic drugs, with subsequent IS responses measured via LC–MS/MS analysis. The mean IS responses were approximately 10,000 for voriconazole and 20,000 for antipsychotic drugs, which were consistent to the data obtained from expert techniques. The accuracy assessment revealed no significant difference among undergraduates, postgraduates, and CME specialists for either analyte (**Figures 2A-2B and 2E-2F**). The precision analysis demonstrated that the voriconazole sample preparation had greater reproducibility (overall IIV: 7.00±3.90) than did the antipsychotic drug preparation (overall IIV: 13.61 (9.86-20.11)). Notably, compared with CME specialists, postgraduate trainees exhibited significantly superior precision for both analytes while outperforming undergraduates specifically in antipsychotic drug preparation (**Figure 2C, 2G**). The consistency among the participants in the different groups demonstrated that the interindividual variation of CME specialists in the performance of voriconazole sample preparation was smaller than that of the other groups but greater than that of the undergraduate and postgraduate groups regarding the preparation of antipsychotic drug samples (**Figure 2D and 2H**).

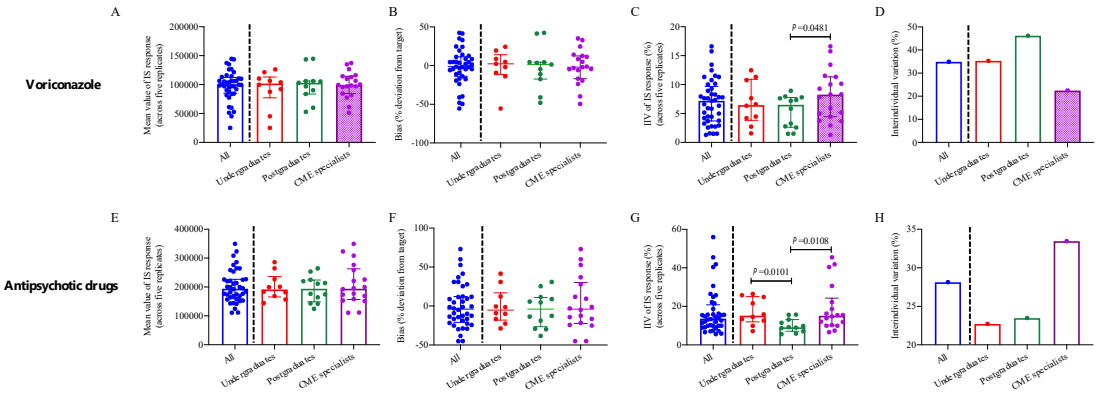

Figure 2 Performance metrics for voriconazole and antipsychotic drug sample preparation across trainee groups.

A, E. Mean IS response across five replicates for voriconazole (**A**) and antipsychotic drug (**E**) sample preparation.

B, F. Accuracy (bias, %) of voriconazole (**B**) and antipsychotic drug (**F**) sample preparation.

C, G. Precision (IIV, %) of voriconazole (**C**) and antipsychotic drug (**G**) sample preparation.

D, H. Interindividual variation (Coefficient of variation, CV, %) in voriconazole (**D**) and antipsychotic drug (**H**) sample preparation among each group.

3.4 Feedback

The satisfaction questionnaire data revealed that 74.41% of the trainees agreed that the training and assessment system inspired their interest in clinical LC–MS/MS, whereas 23.26% held neutral attitudes, and 1 (2.33%) person disagreed. Interestingly, 97.68% of the participants thought the system was helpful in improving their hands-on skills, and 2.32% held neutral attitudes. A total of 86.05% of the trainees did not think the system would increase the burden of their clinical laboratory rotation. Furthermore, no significant differences were observed among the three groups of trainees in terms of the above items (**Figure 3**).

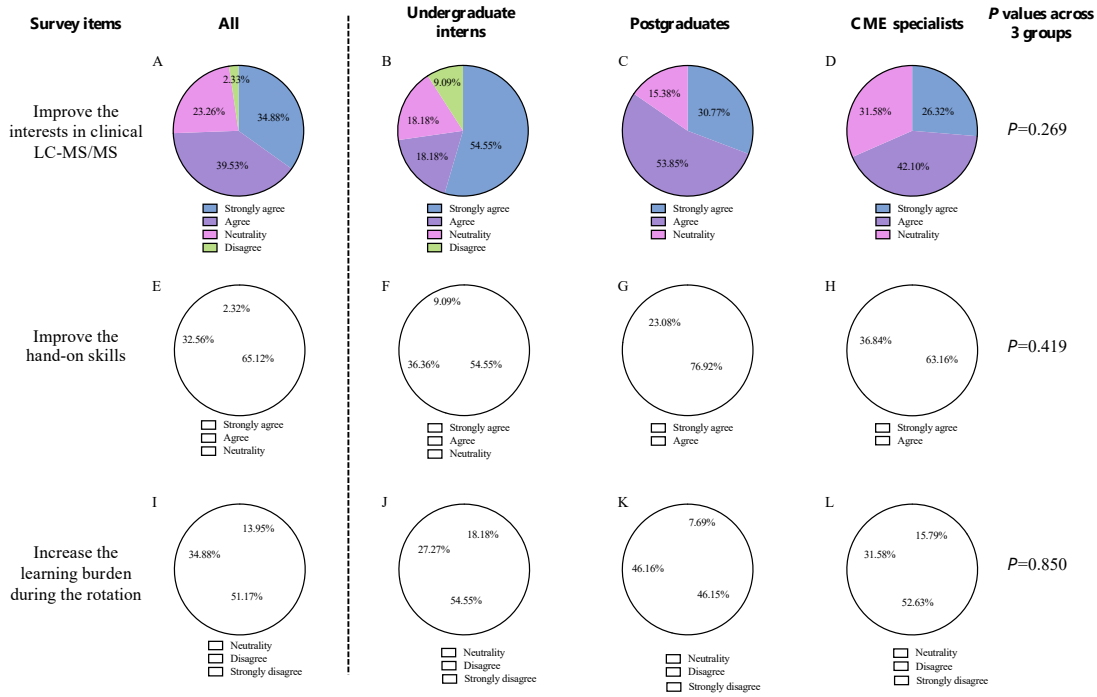

Figure 3 Trainee satisfaction and perceived outcomes of the OJT program.

A-D. Proportions of trainees who believed that the OJT program increased their interest in clinical LC–MS/MS are shown for all participants (**A**), undergraduate interns (**B**), postgraduates (**C**), and CME specialists (**D**).

E-H. Proportions of trainees who believed that the OJT program improved hands-on skills for all participants (**E**), undergraduate interns (**F**), postgraduates (**G**), and CME specialists (**H**).

I-L. Proportions of trainees who believed that the OJT program did not increase their learning burden during the rotation for all participants (**I**), undergraduate interns (**J**), postgraduates (**K**), and CME specialists (**L**).

Note: Data derived from post-training surveys administered via Questionnaire Star. Response categories for each survey item consist of 'Strongly agree', 'Agree', 'Neutrality', 'Disagree', and 'Strongly disagree'.

3.5 Problem identification

We identified several key factors that may contribute to the imprecision and inaccuracy in manual LC–MS/MS sample preparation after posttraining discussions with participants. First, the participants were unfamiliar with the physical characteristics of organic solutions, resulting in inaccurate pipetting of organic solutions, which was believed to be one of the main factors affecting the accuracy and precision of the participants' sample preparation process. Second, insufficient vortex mixing may decrease the sample extraction efficiency, thereby leading to a low IS response value and low accuracy. Third, the volume of transferred supernatants for further concentration was not adequate because of the insufficient hands-on training in this type of transfer.

4. Discussion

OJT refers to a practical learning approach where trainees or employees acquire new competencies, skills and knowledge directly within their work environment through hands-on tasks and mentorship. It emphasizes learning by doing, enabling trainees to adapt to real-world workflows and tools while performing their actual job responsibilities[12, 13]. In this study, we adopted the OJT method by immersing trainees in real LC–MS/MS sample preparation tasks, aiming to help them to gain contextual understanding and quickly integrate tools, processes, and workplaces. We found that this practical educational activity was effective and well accepted by most participants. The medical students and CME specialists included in this study were satisfied with this learning and training process, which was believed to provide valuable practical experience for their future practice in LC–MS/MS. Application of quantitative competency metrics (IIV and Bias) enables timely performance assessment and provides trainees with actionable insights, which helps them understand how their actions align with the expected outcomes and increasing their confidence and motivation. In addition, Supervisors can also identify strengths and areas for improvement and provide timely intervention to prevent the reinforcement of incorrect operations[14].

When implementing an LC–MS/MS practical training and assessment system, several critical factors must be considered. Among these factors, pipetting stands out as a foundational skill that significantly impacts the success of experiments, particularly in sensitive workflows[15]. As the first step in the experimental process, pipetting accuracy and consistency directly influence the preparation of samples, reagents, calibrators, and quality control materials, ultimately affecting the reliability of the LC–MS/MS results. Therefore, all participants in this study were required to complete a pipetting proficiency test using fluids of varying viscosity prior to the formal LC-MS/MS sample preparation. The results revealed that the participants demonstrated lower accuracy and precision when pipetting organic solutions (dichloromethane and MBTE) than when pipetting water, which is consistent with Matthew L Crawford *et.al'*s findings [16] . As anticipated, larger volumes generally resulted in better precision, similar to trends observed in automated systems[17] [18]. Post-training analysis demonstrated that inexperience, poorly seated tips, and excessive carryover contribute to the imprecision and increased pipetting variability of trainees. These findings highlight the need for supervisors to emphasize specific precautions when trainees pipette organic solutions. Manual pipetting proficiency testing should be implemented as a prerequisite in clinical LC-MS/MS analysis. On the basis of the testing results, supervisors can also determine whether additional pipetting training, especially for organic solutions, is necessary for individual participants to ensure optimal performance.

A second critical consideration is the effectiveness of target analyte extraction and the selection of appropriate evaluation metrics. Previously, a series of training and competence evaluation programs in LC-MS/MS were proposed and conducted in other laboratories. For example, Judith A. Stone et al. provided a detailed list of competencies for personnel in LC–MS/MS diagnostic laboratories [6]. Association for Diagnostics and Laboratory Medicine (ADLM) comprehensively discussed the four stages of training and competence for processes and procedures specific LC-MS/MS with detailed training checklist and competence assessment documents provided [19]. However, these studies are more theoretically related and mainly covered in programs for staffs, clinical chemistry fellows and pathology residencies, the specific implementation guidelines and the programs tailored for short-term rotation students are lacking. Here, we refined the detailed steps and adopted preprocessing for voriconazole and antipsychotic drugs, which involves upper-phase extraction and lower-phase liquid-liquid extraction methods, respectively. It provides diverse technical training opportunities to improve trainees' hands-on competencies in LC-MS/MS sample preparation. In addition, we introduced IS response as the readout and the primary competency metric for assessing the accuracy and precision of individual performance. According to China's industry standard, we set the clear competency assessment criteria by integrating IIV and bias values of IS response. These quantitative metrics enable an actionable, real-time feedback to trainees, significantly enhanced the standardization and objectivity of competency assessment.

A third critical consideration is the integration of feedback loops. In our pilot implementation of the LC-MS/MS training program, competency assessments were complemented with real-time troubleshooting discussion and post-training surveys, creating a closed-loop system for continuous improvement. More than two-thirds of the trainees believed that the training practice increased their interest in LC–MS/MS and significantly improved their practical skills without adding an additional burden to their study schedules. This learner-centric approach aligns with the principles of effective training program design, as highlighted by Kirkpatrick's four-level training evaluation model, which emphasizes learner satisfaction and skill acquisition as key indicators of success[20]. As a consequence, our framework not only enables trainees receive immediate metrics alongside supervisor observations, but also fosters trainees' self-correction and iterative learning. Such bidirectional feedback bridges the gap between competency assessment and practical application.

In addition, to explore the feasibility of this OJT program, we conducted the pilot stratified cohort

analyses on 43 trainees from our center. Interestingly, by comparing the training performance of undergraduates, postgraduates and CME specialists, we observed that postgraduates demonstrated the best performance in both training programs, as evidenced by the lower CV values for voriconazole and antipsychotic drugs. In contrast, CME specialists presented significantly higher CV values than postgraduates did. The data revealed that prior hands-on experience may predict the competency of LC-MS/MS sample preparation. Postgraduates, who are actively engaged in clinical and basic research, have honed their experimental skills, whereas undergraduates and CME specialists often lack comparable experience. Moreover, the widespread reliance on automated instruments has reduced opportunities for hands-on practice in clinical settings, which may explain the poorer performance of CME specialists. These results indicate that stratified educational training programs should be implemented to address the potential bias from hands-on experience disparities, thereby ensuring the personalized and effective competency training across all personnel.

There are several limitations in our study. First, the single-center design and restricted sample size limited the generalizability of the findings. The participants were recruited exclusively from one academic medical center and the uneven distribution of subgroups potentially introducing selection bias and reduced statistical power. Larger, multi-center validation are required in future studies. Second, we did not reevaluate the practical performance of trainees after training, which should be considered for the future implementation of training and evaluation systems. Third, we focused only on developing training and evaluating LC–MS/MS sample preparation; we did not cover all the aspects of LC–MS/MS competency assessment, such as troubleshooting instrument errors or interpreting complex chromatograms.

5. Conclusions

This study establishes a competency-driven OJT framework that aims to improve the practical skills of trainees in LC-MS/MS sample preparation. By integrating quantitative metrics with real-time feedback, our program transcends subjective evaluations and standardizes competency assessment. It has important educational implications for other complex, error-prone workflows in clinical laboratory medicine, such as flow cytometry, molecular diagnostics and immunoassays. For instance, pipetting accuracy and precision metrics could be extended to flow cytometry pipetting workflows, while IS-like normalization could be adapted for internal controls in molecular diagnostics. Furthermore, the data on stratified training highlights the need for personalized programs tailored to prior experience, which is vital for optimizing competency development in rotating students across disciplines like clinical microbiology and histopathology. This LC-MS/MS training and assessment system not only bridges the gap between theoretical knowledge and practical proficiency but also aligns with global calls for standardized, data-driven training framework establishment. Future work should focus on increasing the sample size and quantitatively assessing the impact of this training framework on students' proficiency in LC–MS/MS workflows.

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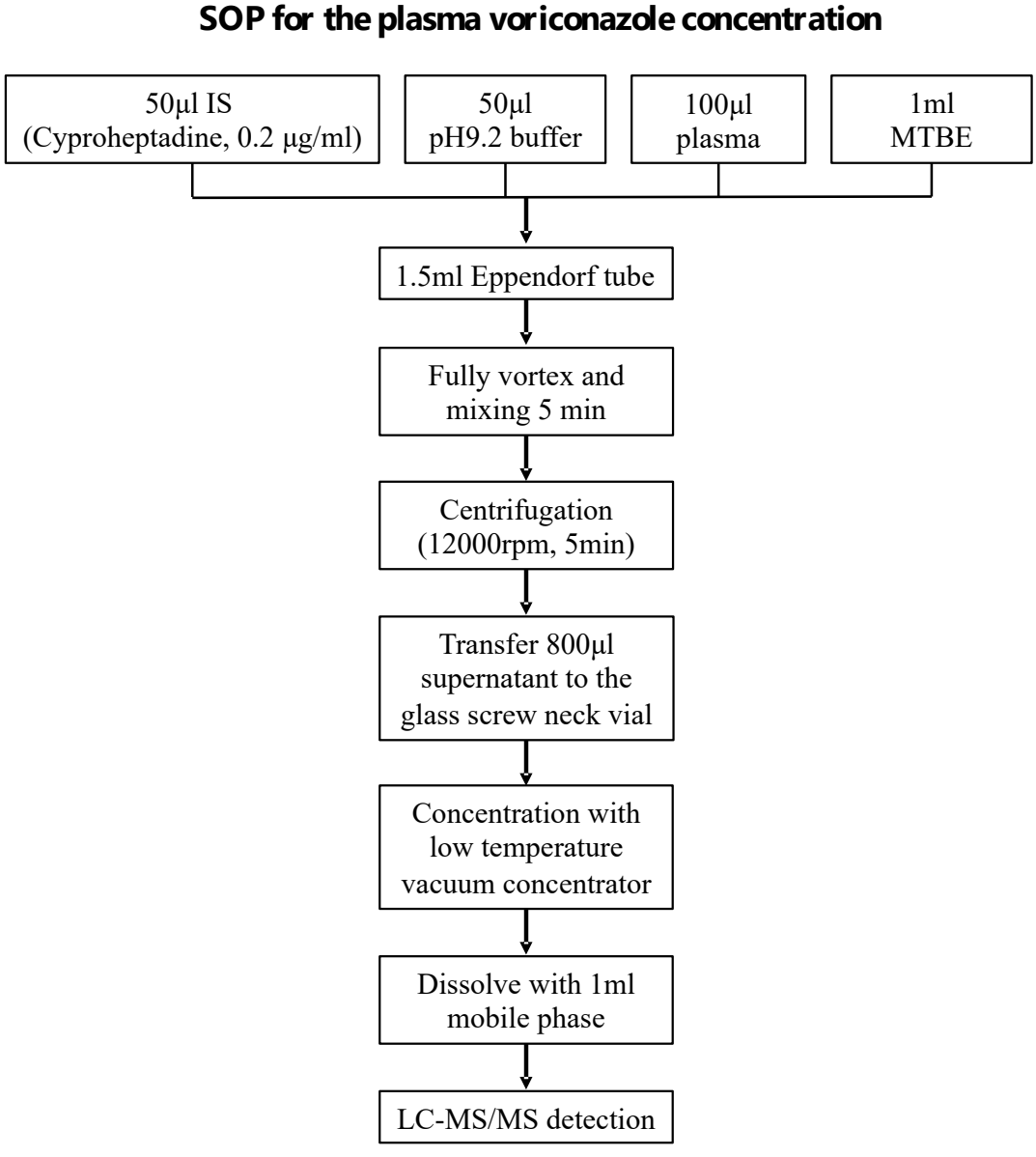
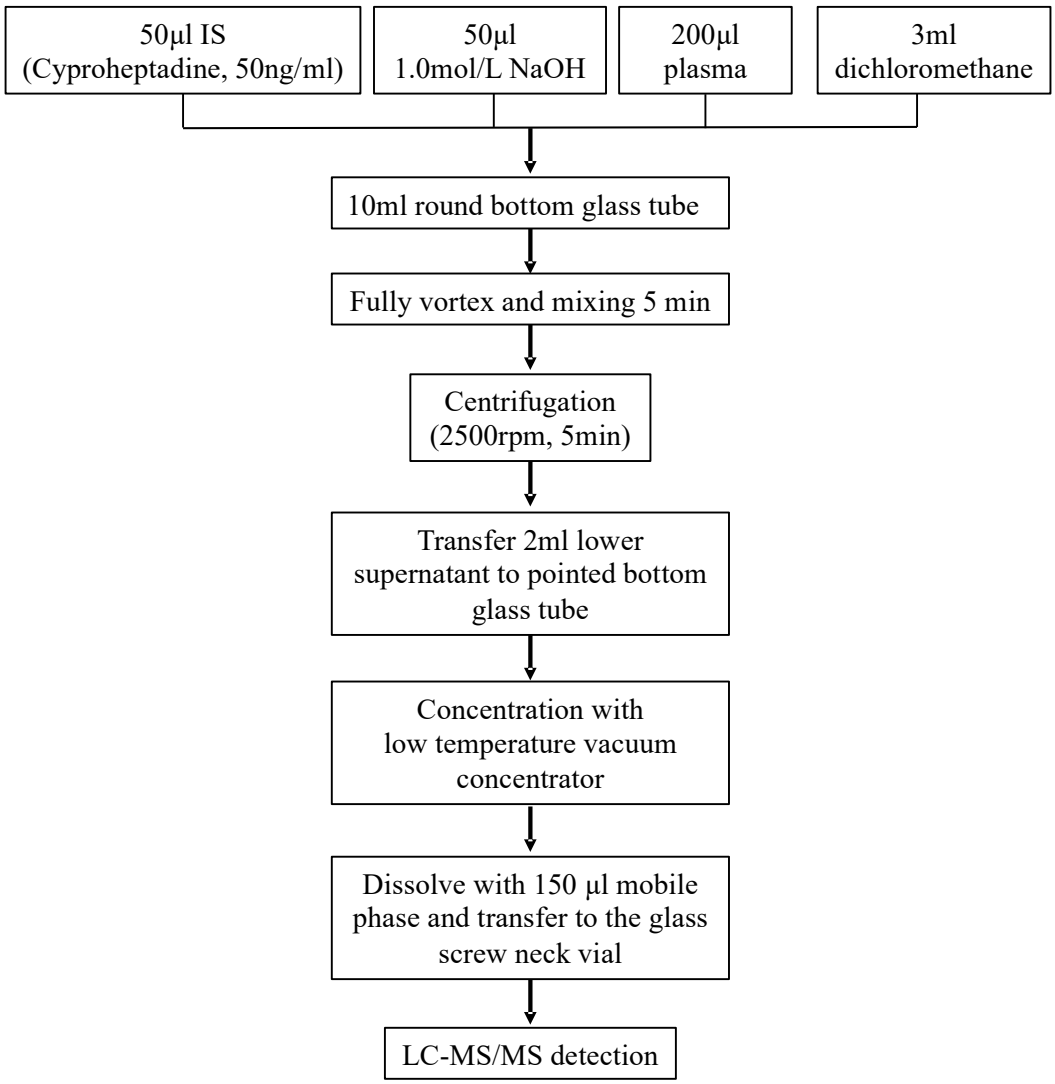
Supplementary materials

Supplementary Table 1 The density and target mass of fluid

	Density	50μl-mass	200μl-mass	1000μl-mass
Water	1 g/cm^3	0.05g	0.20g	1.00g
MTBE	0.74 g/cm^3	0.037g	0.148g	0.740g
Dichloromethane	1.325 g/cm^3	0.06625g	0.265g	1.325g



Supplementary figure 1 The sample preparation SOP for plasma voriconazole concentration



Supplementary figure 2 The sample preparation SOP for plasma antipsychotic drugs concentration