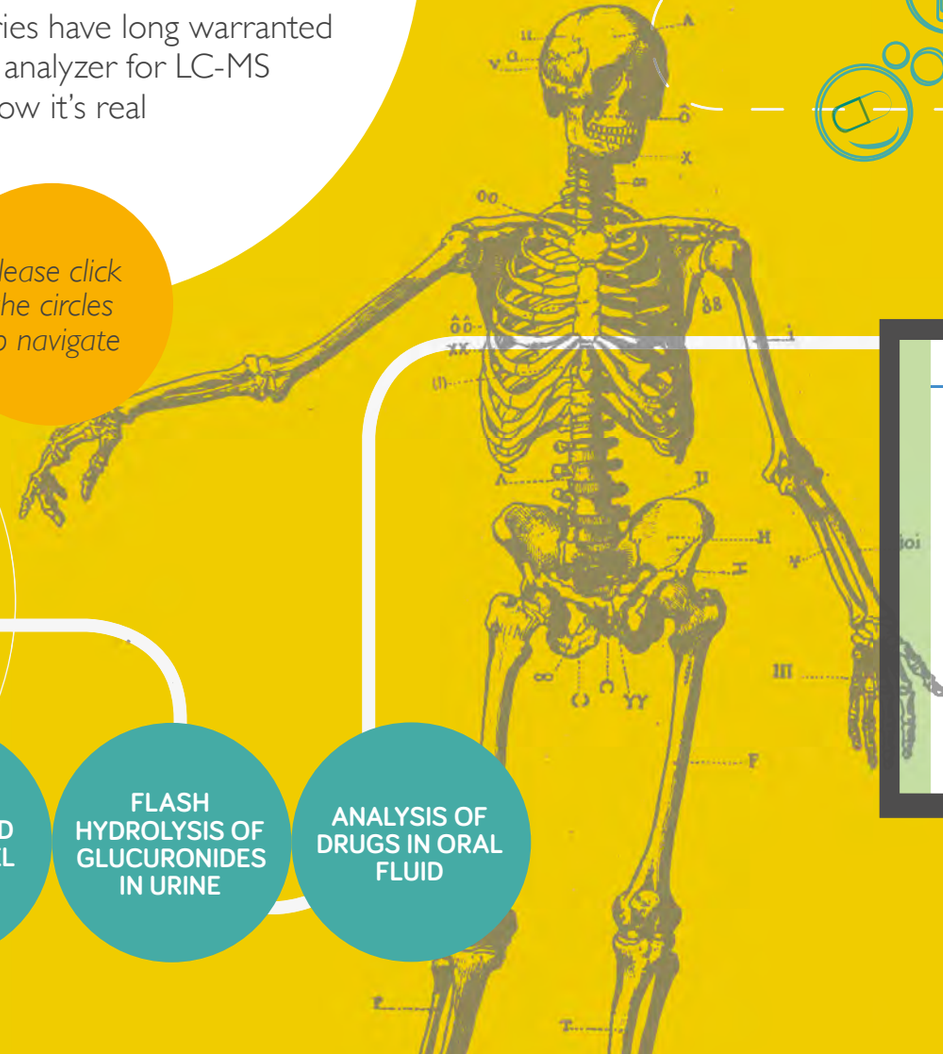
Innovation in Automation

Clinical laboratories have long warranted a walk-away analyzer for LC-MS – now it's real

Please click the circles to navigate



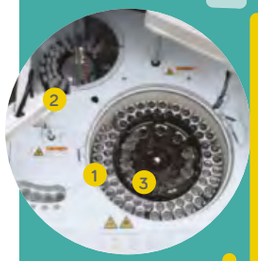
APPLICATION NOTES

- EVALUATION OF HEMOLYSATE AS SAMPLE
- ANALYSIS OF 25-OH VITAMIN D2/D3 IN SERUM
- DETERMINATION OF SIX ANTIARRHYTHMIC DRUGS IN PLASMA
- QUANTIFICATION OF AMIODARONE FROM WHOLE BLOOD
- A 10-STEROID SERUM PANEL
- FLASH HYDROLYSIS OF GLUCURONIDES IN URINE
- ANALYSIS OF DRUGS IN ORAL FLUID

Meet CLAM:

Clinical Laboratory Automation Module

Features



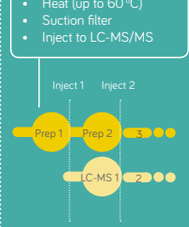
Workflow

1. Load samples
2. Load disposable vials
3. Load reagents
4. Select analysis and START

(5) Parallel preparation and LC-MS/MS analysis

Handling capability:

- Transfer sample to vial
- Add reagent(s)
- Vortex
- Heat (up to 60 °C)
- Suction filter
- Inject to LC-MS/MS



Calibration curve,QC monitoring and data reporting

Raw data inspection as required

Flexibility

Adopting Existing Methods

Reagents are commercially available to assist LC-MS/MS TDM assays, designed to work on the same system

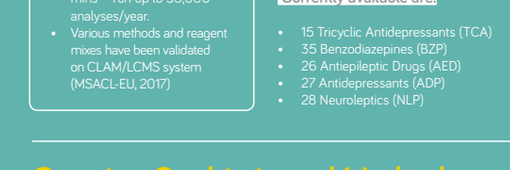
- These methods use the same analytical column and mobile phases, so there is no need to reconfigure the HPLC when switching to a different panel.
- LC-MS run-time: 2.8-7.5 mins – run up to 35,000 analyses/year.
- Various methods and reagent mixes have been validated on CLAM/LCMS system (MSACL-EU, 2017)

Flexible Configuration

CLAM | HPLC or UHPLC | Any LC-MS model

Currently available are:

- 15 Tricyclic Antidepressants (TCA)
- 35 Benzodiazepines (BZP)
- 26 Antiepileptic Drugs (AED)
- 27 Antidepressants (ADP)
- 28 Neuroleptics (NLP)



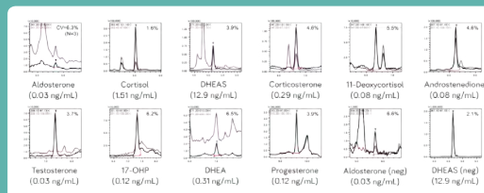
Creating Sophisticated Methods

CASE STUDY 1: Serum steroid panel automated by CLAM/LC-MS (Page 14)

- Panel of 10 steroids, including aldosterone and testosterone that require high sensitivity.
- LLOQ of aldosterone was 0.03 ng/mL, 1/7 of reference level.

CASE STUDY 2: Urine sample analysis with flash enzymatic hydrolysis (Page 16)

- Flash hydrolysis at elevated temperature for 10 min enables high recovery of drug compounds.
- 60 samples in six hours throughput.



MRM Chromatograms and repeatability of 10 Steroids at LLOQ

Benefits

Fully Automated, Wholly Integrated LC-MS Platform for Clinical Research

Less Work & No Errors

- Lower implementation barrier
- Reduced labor cost
- Increases efficiency of existing workflow
- More assays on one system to increase turnover

Run all on CLAM to expand laboratory capability

- Anti-arrhythmics
- Anti-epileptics
- Immunosuppressants
- STAT samples of vitamin D

Manual sample preparation	vs.	CLAM-assisted workflow
• New assays require time and effort to optimize the operational workflow to minimize idle time.	Optimization	• 'Sample to result' process provided by CLAM/LC-MS system minimizes the need for local optimization.
• Implementing a new LC-MS assay warrants an additional member of staff to be assigned and requires sufficient training by highly-skilled operators.	Implementation	• Anyone can operate the CLAM, so work can be shared, reducing workforce expansion. Barriers to implementing new LC-MS assays are lowered.
• Labor accounts for more than 60% of the cost of test results.	Labor	• CLAM/LC-MS system saves labor costs associated with sample preparation, and reduces contamination and hazard risk.
• Emergency samples cause interruption of routine sequences and complicate labor management	Management	• CLAM workflow is sequential and random-access - emergency (STAT) samples have minimal impact on productivity

Recommended applications

1. **Starting a new high-price test, e.g., drug-of-abuse testing, steroid panel.**
   - **Highly recommended:**
     - ✔ Ease of SOP establishment
     - ✔ Good reproducibility from start
     - ✔ Calculable return
2. **Backup system for an existing high-throughput assay e.g., vitamin D**
   - **Recommended**
     - ✔ Accommodation of urgent samples to make routine sequences more efficient.
     - ✔ Increased capacity without increasing labor cost.
3. **Starting low-price tests that are currently outsourced to external laboratories**
   - **Marginally recommended**
     - ✔ CLAM/LC-MS simplifies running various tests on the same system
4. **Combination of 2 and 3**
   - **Very highly recommended**

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Applications of CLAM

The CLAM system has been tested in a wide variety of biomedical applications. A full list (as of March 2018) is given in the table below, and seven⁺ explored in more detail on pages 8–19.

*LCMS configuration

A: online-SPE / isocratic / APCI (switching valve + 2 pumps + APCI ion source)
B: online-SPE / isocratic / ESI (switching valve + 2 pumps + ESI ion source)
C: online-SPE / binary gradient / ESI (switching valve + 3 pumps + ESI ion source)
D: direct injection / binary gradient / ESI (2 pumps + ESI ion source)

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Publication title	Method adopted from		Target compounds	Tested with real sample	LC-MS configuration compatibility*				Recommended LC-MS model
					A	B	C	D	
⁺Fully automated LC-MS/MS analysis of 25-OH Vitamin D2/D3 in serum	RECIPE ClinMass® Complete Kit, MS7000		25-OH Vitamin D2 and 25-OH Vitamin D3	Yes	✔				LCMS-8050
⁺Evaluation of hemolysate as sample in automated sample preparation for the determination of immunosuppressive concentrations by LC-MS/MS – Tacrolimus example	RECIPE ClinMass® Complete Kit, MS1000		Tacrolimus, for proof-of-concept	Yes		✔	✔		LCMS-8040
⁺Fully automated LC-MS/MS determination of six antiarrhythmic drugs in plasma	Homebrew method		Amiodarone, Desethylamiodarone, Bepridil, Flecainide, Pilsicainide, Cibenzoline, Mexiletine	Yes				✔	LCMS-8050
⁺Fully automated online sample preparation AND quantification of Amiodarone from whole blood using CLAM-LCMS	Homebrew method		Amiodarone, Desethylamiodarone	Yes			✔		LCMS-8050
⁺A 10-steroid serum panel on LC-MS/MS integrated with fully automated sample preparation	Homebrew method		Aldosterone, Androstenedione, DHEA, DHEAS, Cortisol, 11-Deoxycortisol, Corticosterone, Testosterone, Progesterone, 17-OHP	Yes			✔		LCMS-8060
⁺Fully automated sample preparation and LCMS analysis of drugs-of-abuse in oral fluid	Homebrew method		Pain management panel of 65 compounds and 62 internal standard, comprising opioids, cannabinoids, drugs of abuse and their metabolites	Yes				✔	LCMS-8050
⁺A novel platform of online sample pretreatment and LC-MS/MS analysis for screening and quantitation of illicit drugs in urine	Homebrew method		Drug screening panel comprising the metabolites of morphine, cocaine and psychoactive drugs	Yes				✔	LCMS-8040
High-sensitivity and simultaneous analysis of psychoactive drugs in serum, whole blood and urine using LC-MS/MS with fully automated pretreatment system	Homebrew method		8 barbiturates, 39 benzodiazepines and 12 tri-/tetra-cyclic antidepressant	Pre-validated with control			✔		LCMS-8050
Quantitation of plasma metanephrine and normetanephrine by derivatization using LC-MS/MS analyzer integrated with fully automated sample preparation device	Homebrew method with new derivatization		Metanephrine and normetanephrine, for proof-of-concept	Proof-of-concept			✔	✔	LCMS-8060
Evaluation of blood lysis procedures prior to automated sample preparation for immunosuppressant assay by LC-MS/MS	Homebrew method		Cyclosporin A, Tacrolimus, Sirolimus, Everolimus	Proof-of-concept		✔	✔		LCMS-8040
Fully automated platform for determination of immunosuppressant drugs in whole blood	Chromsystems MassTox® Kit, ref.93900		Cyclosporin A, Tacrolimus, Sirolimus, Everolimus	Pre-validated with control		✔	✔		LCMS-8050
Fully automated sensitive determination of immunosuppressant drugs in whole blood, using high quality internal standardization	Alsachim Dosimmne® Kit,		Cyclosporin A, Tacrolimus, Sirolimus, Everolimus	Pre-validated with control		✔	✔		LCMS-8050
Therapeutic drug monitoring of antibiotics in plasma: a novel, seamlessly automated LC-MS solution to increase sensitivity, specificity and routine throughput effectiveness	ALIFAX TDM Antibiotics Kit, ref. LC79010		Ciprofloxacin, Levofloxacin, Linezolid, Vancomycin, Gentamicin, Streptomycin, Amikacin, Teicoplanin, Daptomycin	Pre-validated with control				✔	LCMS-8060
Fully automatized LC-MS/MS analysis of neuroleptics in plasma using a novel sample preparation system	Chromsystems MassTox® Kit, ref.92111, 92913, 92914		Two separate methods (using the same configuration, column and mobile phase) for 11 and 13 compounds of neuroleptic drugs	Pre-validated with control				✔	LCMS-8045
Fully automated platform for determination of antiepileptic drugs in serum	RECIPE ClinMass® Complete Kit, MS9000, MS9200		Three separate methods (using the same configuration, column and mobile phase) for covering 26 antiepileptic drugs, 32 benzodiazepines and 15 tricyclic antidepressants	Pre-validated with control				✔	LCMS-8060
Fully automated platform for determination of benzodiazepines in serum	RECIPE ClinMass® Complete Kit, MS9000, MS9500			Pre-validated with control				✔	LCMS-8060
Fully automated platform for determination of tricyclic antidepressants in serum	RECIPE ClinMass® Complete Kit, MS9000 & MS9100			Pre-validated with control				✔	LCMS-8060
⁺Fully-automated sample preparation with flash hydrolysis of glucuronides in urine with LC-MS/MS quantification	Homebrew method with Kura BGTurbo™ enzyme		Mixture of 35 drug compounds, comprising opioids and drug-of-abuse	Proof-of-concept				✔	LCMS-8050
Determination of unbound urinary amino acids incorporated with creatinine normalization by LC-MS/MS method with CLAM-2000 online sample pretreatment	Homebrew method		20 proteinogenic amino acids, citrulline, ornithine and creatinine	Pre-validated with control				✔	LCMS-8040
Evaluation of an automated LC-MS/MS system for analyzing hydrophilic blood metabolites	LC/MS/MS Method Package for Primary Metabolites ver.2		Using the method covering 97 primary metabolites 46 were detected	Proof-of-concept				✔	LCMS-8040

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Evaluation of Hemolysate as Sample in Automated Sample Preparation for the Determination of Immunosuppressive Concentrations by LC-MS/MS – Tacrolimus

A study conducted at Department of Medical Biochemistry and Laboratory Medicine, Merkur University Hospital, Zagreb

By Matea Zoric, Ida Taradi, Katarina Grdisa, Katarina Kajic and Sonja Perkov

Abstract

In order to make the LC-MS/MS assay for immunosuppressants more amenable to automated sample preparation, the study evaluated hemolysate as an alternative sample to whole blood. No statistically or clinically significant difference was observed between the analytical results (n=42) obtained with manual whole blood sample preparation and corresponding hemolysates for the LC-MS/MS measurement of tacrolimus.

Introduction

Manual whole blood sample preparation for liquid chromatography tandem mass spectrometry (LC-MS/MS) for simultaneous quantification of cyclosporine, tacrolimus, sirolimus, and everolimus is currently the method of choice in most laboratories. CLAM-2000 is a fully-automated sample preparation module coupled directly to LC-MS/MS analysis. However, it has no ability for whole blood homogenization before sampling, which poses a reproducibility challenge for the measurement of immunosuppressants that are largely bound to cytoplasmic proteins in erythrocytes. In order to evaluate the suitability of hemolysate as an alternative sample, analytical results obtained with manual whole blood sample preparation versus its hemolysates were compared.

Methods

Tacrolimus concentrations were measured by an on-line SPE LC-MS/MS method (LCMS-8040 and RECIPE ClinMass® LC-MS/MS Complete Kit for Immunosuppressants) in 42 whole blood patient samples collected 12 hours post-dose. The analytical method used was accredited according to ISO 15189 and monitored by internal and external quality assurance programs (Referenzinstitut für Bioanalytik, Germany) since 2015. The long-term evaluation of the obtained results showed excellent results with mean bias of 3 percent according to reference values.

For hemolysate preparation, aliquots of 500 µL of whole blood samples from the same patients were used as an alternative sample, by the freeze–thaw method at -80 °C for 30 minutes (since -20 °C did not cause complete hemolysis for all patients even after 3 hours freeze). Samples were thawed at room temperature, mixed for 10 seconds on vortex, centrifuged for 10 minutes at 3000 rpm and then tacrolimus concentrations were measured using the same method as in the whole blood samples. Hemolysis was assessed qualitatively (visually) and quantitatively (plasma hemoglobin was measured spectrophotometrically and then compared to whole blood hemoglobin). The difference and correlation between obtained results were assessed using t-test and Passing–Bablok regression analysis in MedCalc statistical program.

Results and discussion

Passing and Bablok regression analysis revealed satisfactory comparison (y = -0.061+ [-0.150 – 0.059] + 0.969[0.938 – 1.038]x) and t-test showed that there was no statistically significant difference (P = 0.780) in obtained results. Mean bias for concentration range

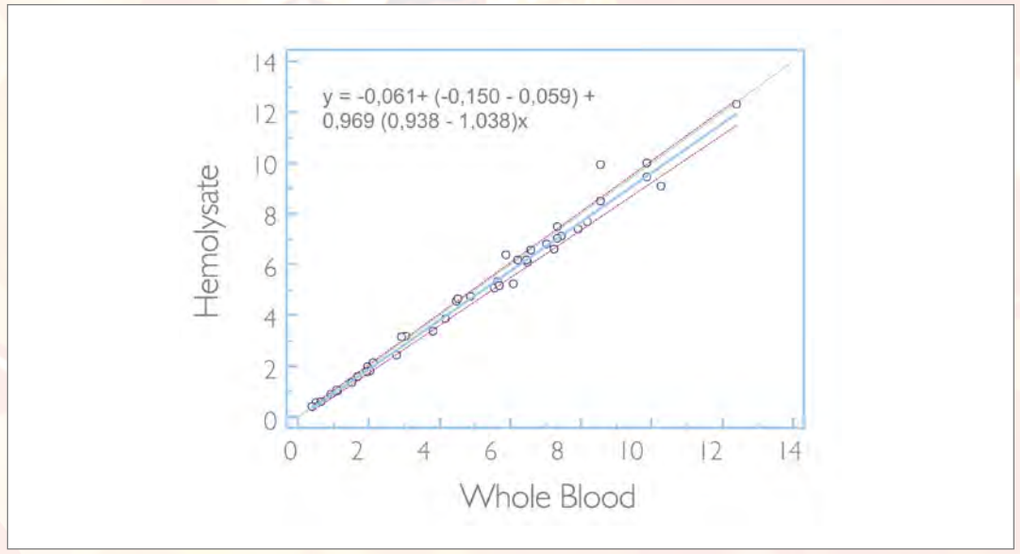

Figure 1. Passing and Bablok regression analysis.

0.5 – 12 µg/L was 0.14 ± 0.17 µg/L (3.8 ± 6.8 percent) with maximum bias of around 1 µg/L (15 percent), which could be considered within biological variation and/or handling errors. However, this should be further evaluated on a larger number of samples.

The obtained results suggested that hemolysate could be a good alternative for whole blood samples for automated sample preparation on CLAM-2000. The automated method could be a great solution for high-throughput therapeutic drug monitoring of immunosuppressive drugs in routine practice and could lead to a decrease in analytical variability and an increase in overall result quality.Still, availability of freezers with the ability to freeze at -80 °C could be an obstacle for implementation of proposed sample preparation principle in routine laboratory work.

Conclusion

Hemolysate produced by a freeze–thaw procedure would be an ideal sample for whole process automation for immunosuppressant determination by the CLAM-LCMS system.

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Fully Automated LC-MS/MS Analysis of 25-OH Vitamin D2/D3 in Serum

A collaboration with the Mass Spectrometry Toxicology Laboratory, Hospital Desio, Italy.

By Daisuke Kawakami, Davide Vecchietti and Maura Brambilla

Abstract

Pre-validated LC-MS/MS assay for 25-OH Vitamin D2/D3 was transferred to automation by CLAM-2000. Results showed excellent precision for both intra-day and inter-day assays, and comparison of the data acquired through manual sample preparation and that by automation was performed using 30 human serum specimens.

Introduction

Vitamin D measurement has become an important component in clinical assays largely because deficiency is associated with a number of disorders, such as rickets, osteomalacia and osteoporosis.

LC-MS/MS has become an essential tool for monitoring the concentration of Vitamin D2/D3 in biological samples due to its high level of sensitivity and specificity; however, manual sample preparation often involves several complicated steps which can introduce error into the results. Additionally, the time-consuming nature of the sample preparation and the large number of samples makes LC-MS/MS a less desirable method. Automated sample preparation has been shown to eliminate human error, as well as increase laboratory efficiency, making LC-MS/MS a feasible method to incorporate in the clinic.

In this study, we investigated the ability to analyze for 25-OH Vitamin D2/D3 by LCMS-8050 coupled with automated sample preparation by CLAM-2000 (For research use only. Not for use in diagnostic procedures) to process large sample sets. This system is seamlessly integrated with the LC-MS/MS system requiring no human involvement after loading the biological samples into the sample chamber. We validated the automated method by using a commercially available test kit ClinMass® LC-MS/MS Complete Kit for 25-OH-Vitamin D2 / D3, MS7000 (RECIPE Chemicals + Instruments GmbH, Germany).

Results and discussion

The calibration curves showed good linearity (R^2>0.999) over a clinical relevant range of 4.10 - 68.5 g/L for 25-OH Vitamin D2 and 4.68 - 77.3 g/L for 25-OH Vitamin D3. The reproducibility (n=7) at three concentrations, including LLOQ, of each compound was excellent (CV<6.5 percent). Results were reproducible on three different days (n=7) at three concentrations as well (CV<7.2 percent).

Comparison of 25-OH Vitamin D3 concentration between manual sample preparation (following RECIPE instructions) and automated sample preparation shows good agreement as highlighted by Passing and Bablok plot and scores (Figure 1).

Conclusion

- Fully-automated sample preparation procedure was found suitable for the quantitation of 25-OH Vitamin D by elimination of all manual preparation steps.
- Automation of the method increases the analytical performance, reduces the risk for human operators and, due to the reduced reagent consumption, reduces the cost of the analysis.

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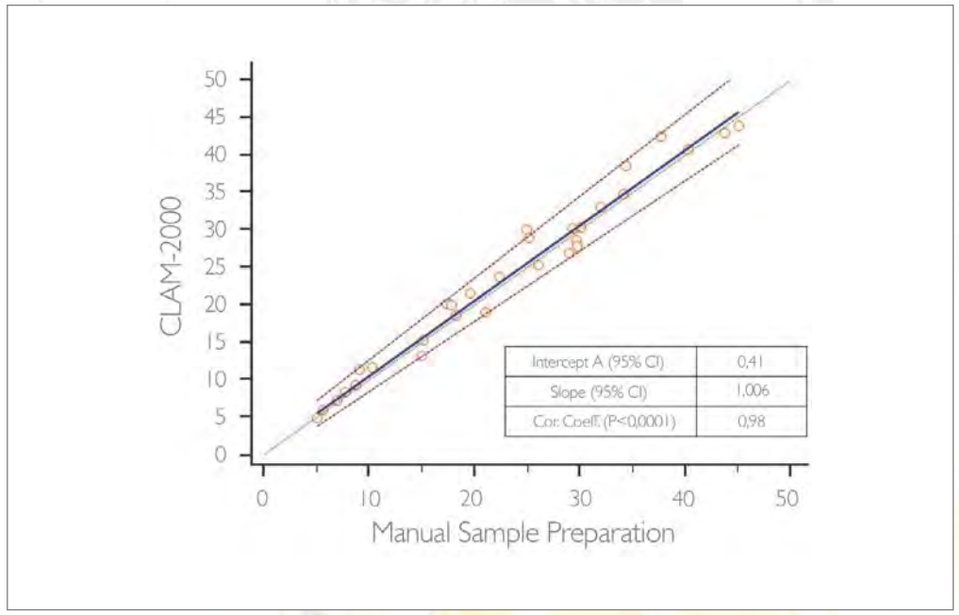

Figure 1. Data correlation between automatic sample preparation (CLAM-2000) and manual method (n=30 human serum samples, plus two reference materials, RECIPE).

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Fully Automated LC-MS/MS Determination of Six Antiarrhythmic Drugs in Plasma

Transferring in-house developed LC-MS/MS assay to automation by CLAM-LC-MS – case study from the National Cerebral and Cardiovascular Center Hospital, Japan.

By Taku Tsukamoto and Daisuke Kawakami

Abstract

Fast and fully automated LC-MS/MS analysis of six antiarrhythmic drugs was developed. Basic assay performance was validated and good accuracy and repeatability was successfully shown. This application demonstrates the ease of LC-MS method transfer where sample pretreatment has previously been conducted manually.

Introduction

Drugs that pose administration management difficulties – such as those with a narrow therapeutic range or large biological variation in pharmacokinetics – require monitoring of circulating concentrations to determine the optimal dose and method of administration for each patient. LC-MS/MS measurement is needed for fast and accurate monitoring of these drugs, especially when multiple drugs are being considered simultaneously. This study achieves a fast and simple workflow of six antiarrhythmic drugs using CLAM-2000.

Methods

Using CLAM-2000, sample pretreatment procedure was conducted by adding 285 µL acetonitrile to 15 µL of plasma, which was then vortexed for 120 seconds before the precipitate was removed by vacuum filtration. The filtrate was seamlessly injected to LC-MS/MS for analysis.

Results and discussion

The CLAM-LC-MS assay for six antiarrhythmic drugs was validated for basic assay performance using control plasma samples. Matrix-matched standard curves were created for the range 50–1500 ng/mL (bepridil, flecainide and cibenzoline) and 100–3000 ng/mL (amiodarone, desethylamiodarone, pilsicainide and mexiletine). Accuracy and precision was evaluated based on the analytical results of the independent set of QC samples. The accuracy was within the acceptable range of 85–115 percent, including the LLOQ sample. Similarly, precision was far below 15 percent RSD and, considering that the whole sample preparation was also repeated for each measurement, good inter-assay repeatability was demonstrated.

Carryover of the CLAM-LC-MS system was evaluated by subjecting blank plasma sample to analysis immediately after the analysis of the highest calibration standard sample. No significant carryover was observed for all drugs with respect to the peak intensity of the LLOQ standard. The results suggest that carryover would not be an issue on this CLAM-LC-MS system for use in real-world samples where a high concentration difference can occur in consecutive samples.

Furthermore, comparison of quantitative results acquired by the CLAM-LC-MS system versus manual sample preparation was performed using a small, real sample set (n=9) and observed no significant bias .

Conclusion

Fully automated LC-MS/MS assays for six antiarrhythmic drugs have been successfully validated for basic performance. This potentially has a high impact for laboratories requiring efficient management of routine and emergency samples.

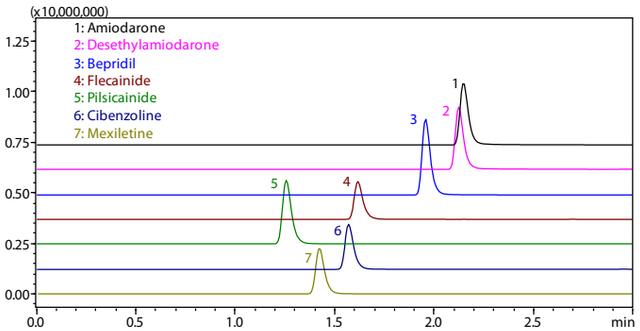

Figure 1. Mass chromatograms of human blood plasma with standard additives

Acknowledgement

We would like to thank pharmacist Yuko Shimamoto of the Pharmacy Division at the National Cerebral and Cardiovascular Center Hospital (National Research and Development Agency) in Japan for her significant cooperation in the investigation provided in this article.

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Fully Automated Online Sample Preparation and Quantification of Amiodarone from Whole Blood Using CLAM-LCMS

Benefits of automation in private testing laboratories – the case of Labor Dr. Wisplinghoff, Germany.

By Christian Bunse and Lars Kröner

Abstract

This study shows the successful online coupling of an automatic sample preparation module (CLAM) with a LC-MS/MS system for accurate and reliable quantification for clinical drugs in whole blood. The system matches with the demands of clinical testing laboratories, providing sample-to-result automation and high efficiency in accepting emergency samples.

Introduction

Amiodarone is an antiarrhythmic drug used to treat and prevent irregular heartbeats. Though very effective and reliable, serious side effects can occur during treatment with the drug. Therefore, this drug has to be monitored during the medication in order to minimize those side effects and their impact on the health of the treated patient.

The method of choice for therapeutic drug monitoring in clinical laboratories is quantification by LC-MS/MS analysis. After a whole blood sample is taken from a patient, ideally a fully automatic analysis is desired, starting with sample preparation before transferring the prepared sample aliquot online to the LC-MS/MS system for subsequent amiodarone quantification.

Methods

Whole blood patient samples were taken and centrifuged at 6,000 rpm (Hettich Rotofix 32A). The obtained blood plasma then was put directly into the CLAM-unit, the barcode of the sample tubes were read automatically, followed by automatic sample preparation and online LC-MS/MS analysis (LCMS-8050).

Results and discussion

Figure 1 shows the full chromatogram of desethylamiodarone and amiodarone in a patient sample. A fast method, wherein chromatographic elution completes in 2.5 minutes, was developed in order to gain high throughput in the processing of clinical samples, which is essential in the field of therapeutic drug monitoring. In addition, the CLAM-2000 provides priority measurement of emergency samples that frequently occur in clinical laboratories.

Peak profiles shown in Figure 2 were obtained, measuring five calibration levels of desethylamiodarone and amiodarone from 0.5 to 3 ng/µL. The linear correlation factor was 0.9994 for both components, indicating very good linearity. Both components were detectable at the lowest concentration of 0.5 ng/µL, with high peak intensity of 750,000 for desethyllamiodarone and 400,000 for amiodarone, respectively.

Figure 3 shows peak profiles and the calculated concentration derived from the calibration curve. Even very low amounts could be detected with high peak intensities. Though measuring in a very harsh matrix, there is nearly no background noise thanks to a well-established CLAM-preparation protocol and the use of a pre-column.

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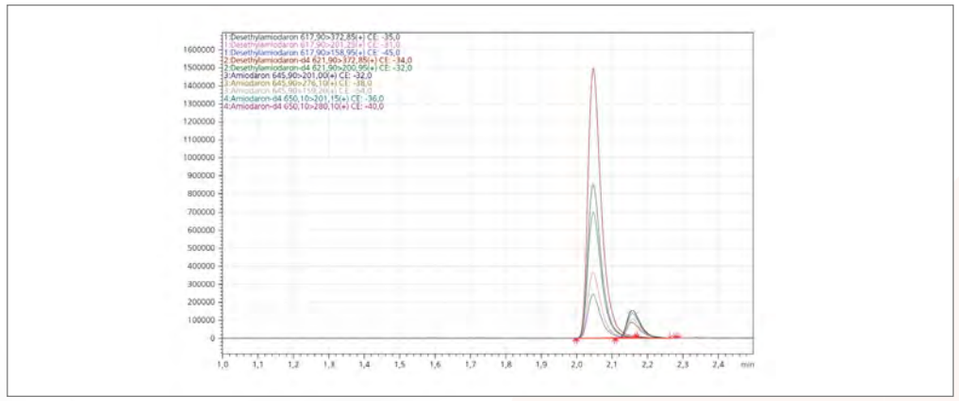

Figure 1. MS chromatogram of patient sample.

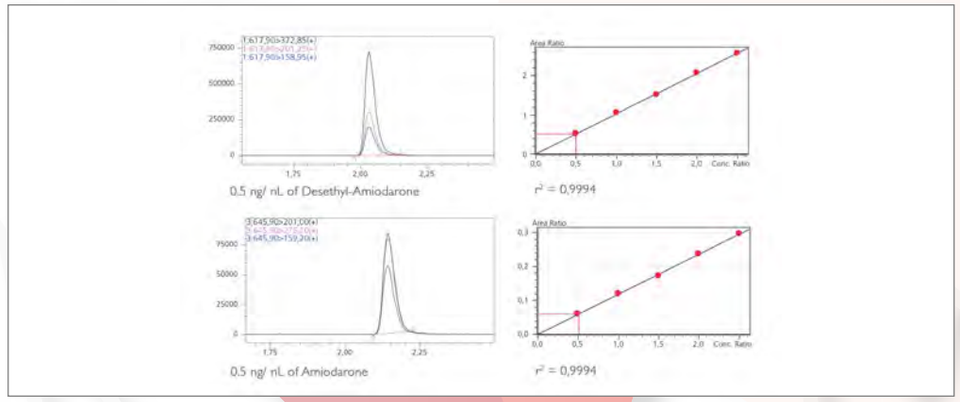

Figure 2. Peak profile at 0.5 ng/µL and calibration curves (0.5-3 ng/µL).

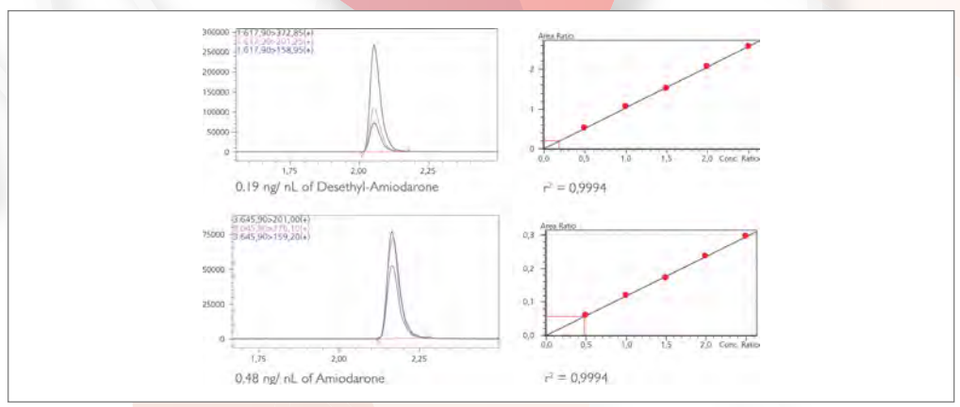

Figure 3. Peak profiles from patient sample.

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A 10-Steroid Serum Panel on LC-MS/MS Integrated With Fully Automated Sample Preparation

The flexibility of CLAM-2000 allows even a cutting-edge application to be transferred to automation.

By Toshikazu Minohata and Daisuke Kawakami

Abstract

A commercially available 10-steroid panel kit was transferred to automation with chromatographic modifications. The same level of sensitivity was achieved with excellent repeatability, while improving the analytical workflow by automation and shortening of sample preparation time.

Introduction

Currently, sample preparation for the detection of steroids in serum by LC-MS/MS involves complex offline extraction methods, such as solid phase extraction or liquid–liquid extraction, all of which require additional sample concentration and reconstitution in an appropriate solvent. These sample preparation methods are time-consuming, often taking an hour or more per sample, and are more vulnerable to variability due to human errors during manual manipulation.

Methods

Certified standards in serum matrix (CHS™ MSMS Steroids Kit, PerkinElmer, USA) were used for 10 of the steroid hormones. (Abbreviations: 17-alpha-hydroxyl-progesterone, 17-OHP; 4-androstene-3,17-dione, androstenedione; dehydroepiandrosterone, DHEA; dehydroepiandrosterone sulfate, DHEAS.)

Sample preparation by CLAM-2000 was conducted as follows:

1. 60 µL of acetonitrile (containing internal standards) added to 30 µL of serum
2. Vortexing for 150 sec
3. Filtration
4. Direct injection of filtrate

We adopted a trap-and-elute online sample cleanup system using the MAYI-ODS column.

Results and discussion

We evaluated this system using calibrator and control serum spiked with 10 steroids contained in the kit, and carried out simultaneous analysis over the following range of concentrations: cortisol (1.51-320 ng/mL), aldosterone (0.03-1.14 ng/mL), 11-deoxycortisol (0.08-18 ng/mL), corticosterone (0.29-62 ng/mL), 17-OHP (0.12-26 ng/mL), androstenedione (0.08-18 ng/mL), DHEA (0.31-65 ng/mL), DHEAS (12.9-2750 ng/mL), progesterone (0.12-26.5 ng/mL) and testosterone (0.03-7.2 ng/mL). The resulting calibration curves had linear regression values of r^2>0.997 for each curve. The inter-assay repeatability (n=3) at seven concentrations, including LLOQ of each compound was excellent (<10 percent RSD). Moreover, as shown on the MRM chromatograms, chromatographic separation of steroids was such that there was no overlap between 17-OHP and DHEA, as well as cortisol and aldosterone.

As the result of system improvement and automation, minimum time requirement for sample preparation was 10-times shorter, for accelerated throughput without compromising assay sensitivity. While conventional methods relying on manual preparation required the removal of organic solvent for better chromatographic resolution, this system utilized the MAYI-ODS column that could trap steroids efficiently even in the presence of organic solvent, while residual proteins and other macromolecules were discarded in the unbound fraction.

Conclusion

We have successfully transferred the LC-MS/MS steroid panel to automation by CLAM-2000 for improving the analytical workflow.

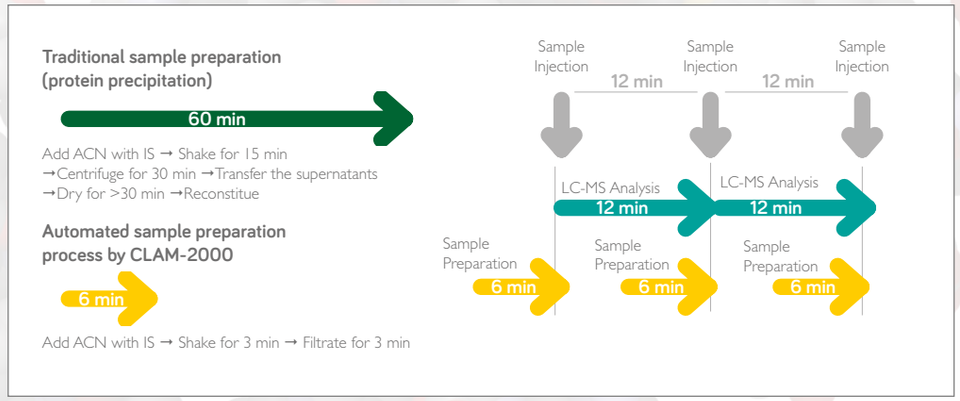
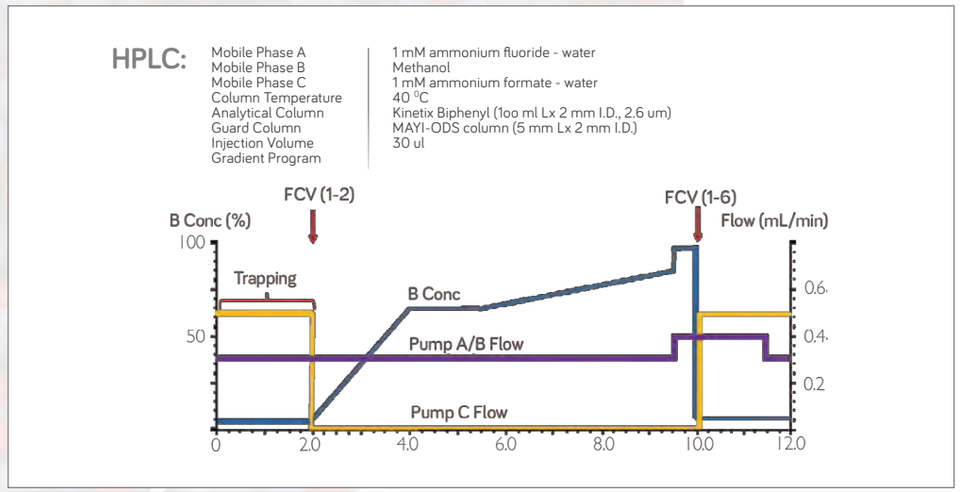


Figure 1. Comparison with a time required for sample preparation.



The results were promising, with anticipated enhancement in accuracy and precision due to elimination of human error associated with manual sample manipulation, though more validation work is needed with a large sample set.

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Fully Automated Sample Preparation With Flash Hydrolysis of Glucuronides in Urine With LC-MS/MS Quantification

Sequential, online sample preparation is the best way to ensure reproducibility of enzymatic reactions.

By Joshua Emory, Nicoletta Bianchini, Brian Feild, and Yves-Vincent Duperron

Abstract

This study used genetically modified beta-glucuronidase (BGTurbo) operating at elevated hydrolysis temperatures (55 °C) and parallel processing by CLAM-2000. This was applied to urinary drug analysis containing glucuronidated drug species, and showed potential to analyze up to 60 samples in six hours.

Introduction

Currently, sample preparation for the detection of glucuronidated drug compounds in urine by LC-MS involves multi-stage manual sample preparation methods, which often leads to manual preparation errors. Typical manual sample preparation of glucuronides can take up to two hours. However, a new enzyme, BGTurbo, is fast enough to make serial flash hydrolysis possible. The CLAM-2000 sample preparation module seamlessly integrates sample preparation, LC separation and MS detection of small molecules in an online platform. Using the BGTurbo enzyme, we developed a serial fully automated flash hydrolysis, sample preparation, and LC-MS analysis method for glucuronides and other drugs of abuse in urine. This method maximizes efficient use of the mass spectrometer – using parallel sample processing of up to four samples simultaneously so that the LC-MS system is running constantly.

Methods

Six glucuronides (morphine 6 beta, codeine 6 beta, buprenorphine 6 beta, oxazepam 6 beta, naltrexone 3 beta, 11-nor-delta 9-THC [Cerillant, Round Rock, TX]) in surine (Sigma Aldrich, St. Louis, MO) were converted to their parent drug forms using a genetically modified beta-glucuronidase enzyme (BGTurbo, Kura Biotec, Puerto Varas, Chile). Online sample preparation and LC-MS/MS analysis was conducted automatically as illustrated in Figure 1. In the investigation for optimum digestion condition, a fixed enzyme:sample ratio of 1:2 (v/v) was used for consistency.

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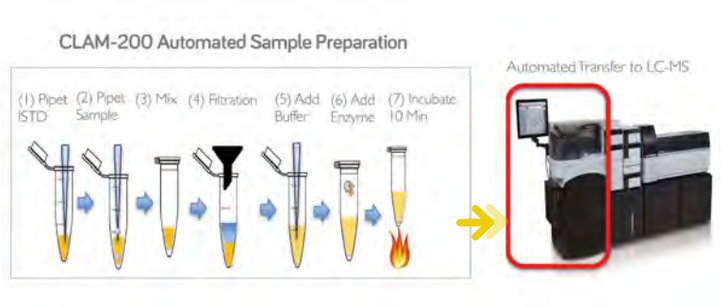

- Methanol, Formic Acid (Sigma-Aldrich, St. Louis, MO)
- Column: 2.1x50mm Raptor Biphenyl (Restek, PA)
- Mobile Phase A (0.1% Formic in Water), Mobile Phase B (0.1% Formic in Methanol)
- Gradient: 10% to 95% Methanol over 5.9 minutes
- LC/MS system: Shimadzu Nexera LC system and a Shimadzu 8050 triple quadrupole

Figure 1. Sample preparation and analysis on CLAM-2000 and LCMS 8050

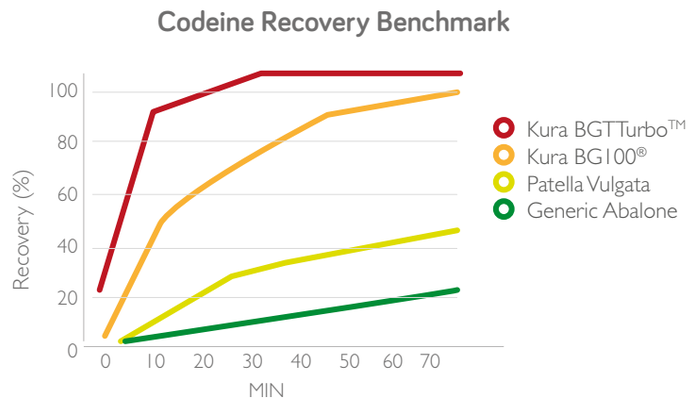

Figure 2: Graphical representation of codeine percent recovery with four different enzymes. BG Turbo exhibits over 85 percent recovery in under 10 minutes.

Results and discussion

Optimization for online sample preparation revealed that many glucuronides, like buprenorphine, hydrolyzed readily at 50 °C, but incubation conditions of 55 °C and 10 minutes were required to effectively hydrolyze codeine-6-beta glucuronide (Figure 2), which is known as one of the most difficult glucuronides to hydrolyze completely. Using the optimum condition as shown, all glucuronides except codeine-6-glucuronide had percentage recoveries greater than 97 percent.

The CLAM-2000 sample preparation module with a Shimadzu LCMS-8050 was used to serially perform enzymatic hydrolysis of glucuronides in a 35-component drug mixture on an LC-MS timescale (5–7 minutes), which allowed the LC-MS system to run nonstop after the first sample. Calibration curves showed $r^2 > 0.99$ and had linear dynamic ranges of 1 – 5000 ng/mL (6-acetylmorphine, oxycodone, MDA, tapentadol and benzoylecgonine), 1 – 1000 ng/mL (codeine, hydromorphone and methamphetamine) and 10 – 5000 ng/mL (morphine and oxazepam). Representative MRM chromatograms at LLOQ are shown in Figure 2.

Conclusion

Fully automated flash hydrolysis, sample preparation and LC-MS analysis of glucuronidated drugs of abuse was integrated to LC-MS using BGTurbo enzyme and a Shimadzu CLAM-2000. The use of the genetically modified beta-glucuronidase operating at elevated hydrolysis temperatures (55 °C) and parallel processing made serial sample preparation and analysis possible, allowing up to 60 samples to be analyzed in six hours. The CLAM-2000 sample preparation module increases sample preparation continuity, laboratory safety and laboratory efficiency while reducing the sample preparation work performed by laboratory personnel.

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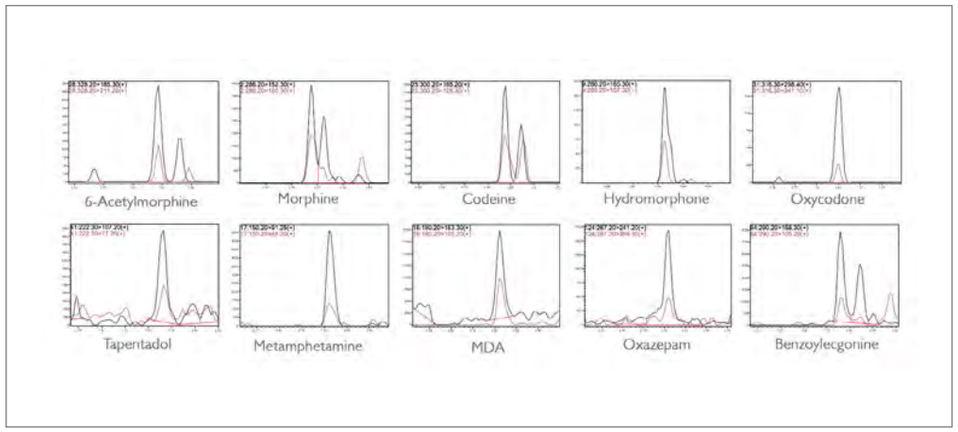

Calibration curves (L1-L9) and MRM chromatograms (L1) for ten drug compounds.

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Fully Automated Sample Preparation and LC-MS Analysis of Drugs in Oral Fluid

Developing a novel and fully automated in-house assay – the case of Captiva Laboratory, NC, USA.

By Nathan DeFreitas, Joshua Emory and Manoj Tyagi

Abstract

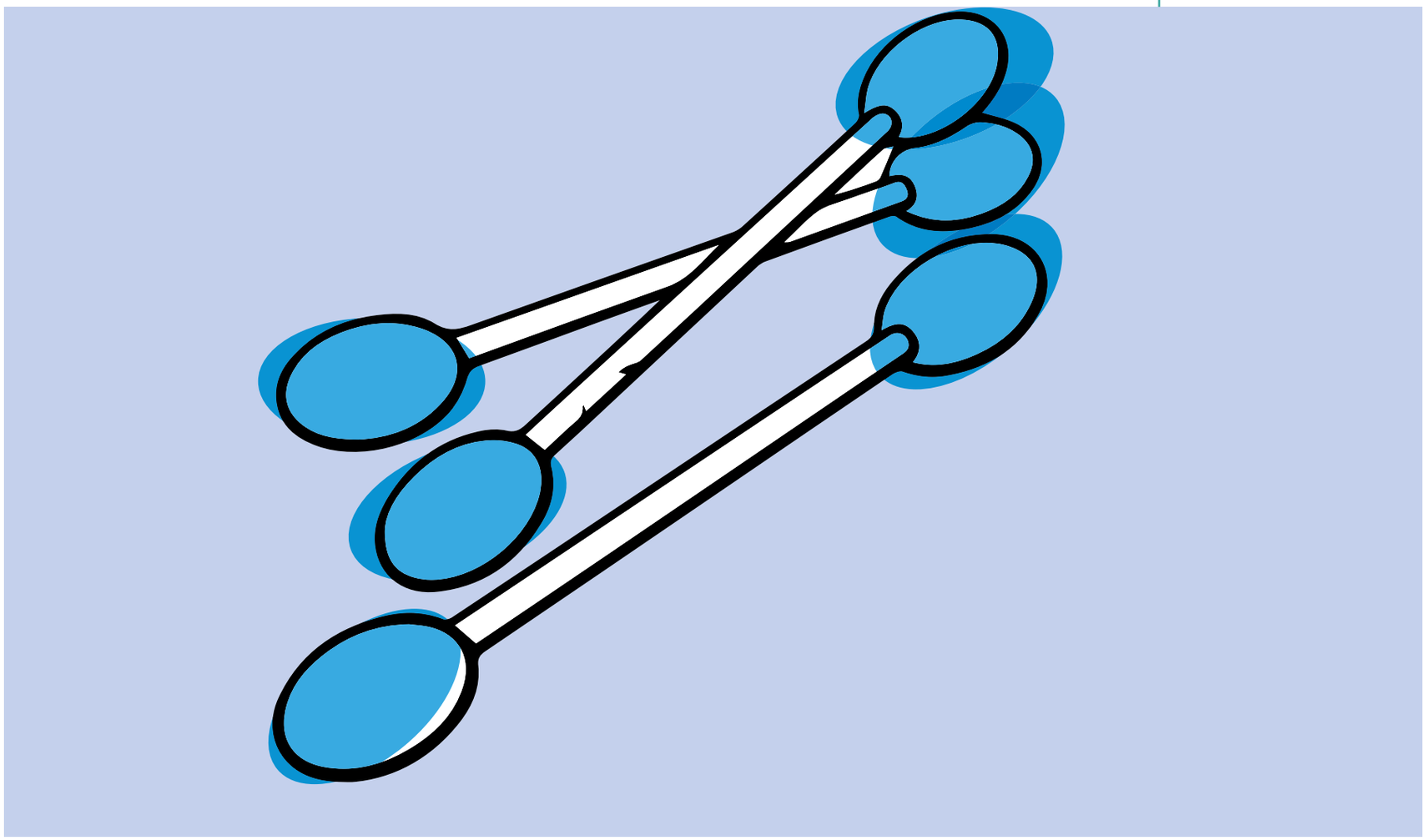
The Clinical Laboratory Automation Module (CLAM-2000) is a fully automated sample preparation instrument that is integrated with a Shimadzu LCMS analyzer. This system has been used for analysis of 122 drugs and deuterated internal standards in oral fluids with detection limits of 2 ng/mL. This system offers high sample throughput, improved efficiency and increased safety for laboratory personnel.

Introduction

Despite recent advances in LC and MS technologies which enable faster and more robust analytical methods, advances in sample preparation for small molecule analysis have been slower to develop. Although there are robotic devices for offline sample preparation, there are no other fully automated/integrated online LC-MS sample preparation modules. The CLAM-2000 sample preparation module seamlessly integrates sample preparation, LC separation and MS detection of small molecules in an online platform. We have developed a fully automated method for sample preparation, LC separation and MS quantification of 122 drugs (including deuterated internal standard) in oral fl¬uid. This system offers reproducibility of less than 10% RSD along with parallel processing for up to four samples to maximize mass spectrometer up time.

Methods

Oral fluid analysis was performed using the CLAM-2000 integrated with a Shimadzu Nexera LC system and a Shimadzu LCMS-8050 triple quadrupole mass spectrometer. A gradient of 10% to 60% Methanol was implemented over seven minutes. All samples, calibrators and quality controls were made in Quantisol oral fluid diluent.



Analytical Method Summary

# Drug Compounds	122 (66 target + 56 IS)
Time for first results	12 mins
Sample to sample time	7 mins
LOQ	2 ng/mL
Average %RSD	7.5%
R^2 values	>99

Table 1. Analytical Method Summary

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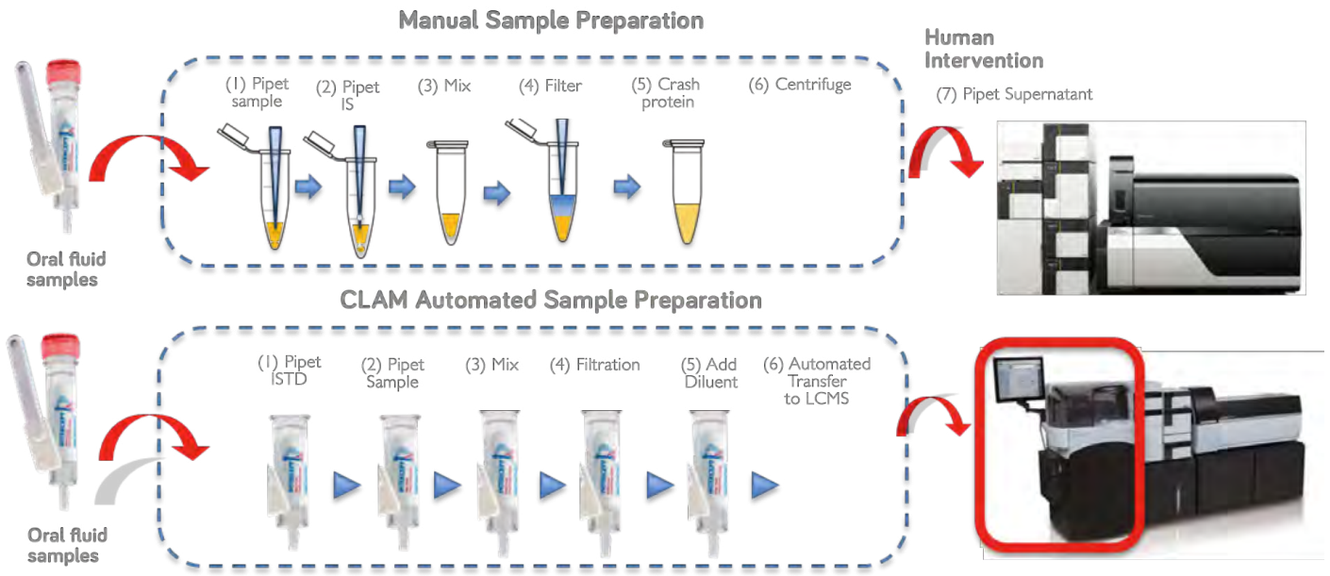

Figure 1. Comparison of manual sample preparation and LCMS analysis and CLAM-2000 fully automated sample preparation and LCMS analysis. The many steps requiring human intervention during manual preparation not only introduces human error but costs more because employees are being paid to do routine tasks that are more easily accomplished by automation.

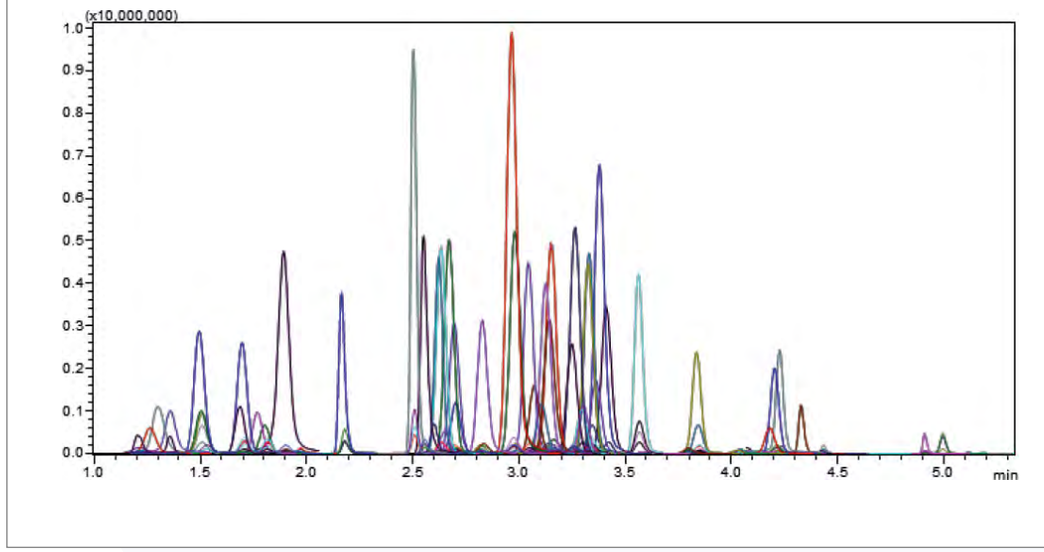

Figure 2: Simultaneous acquisition of 66 compounds in five minutes

Result and discussion

Fully automated sample preparation, LC separation and MS analysis of 122 drugs and deuterated internal standards were performed using the CLAM/LC-MS system. No sample preparation was performed by lab personnel aside from loading the oral fluid collection devices into the instrument carousel (Figure 1). After the first sample preparation and LCMS analysis, which takes eleven minutes, the sample to sample analysis time is equal to the LCMS method time (seven minutes). Figure 2 shows the MRM chromatogramsof 66 compounds, demonstrating excellentseparation in five minutes. Calibration curves for the drugs of abuse exhibited R2 values ≥ 0.99 and limits of quantification ranged from 2 ng/mL for most compounds to 7.5 ng/mL for Amphetamine. This novel sample preparation method achieved percent relative standard deviations of ~10% or less for all compounds (Table 1).

Conclusion

The fully automated sample preparation and analysis of over one hundred drugs and internal standards was performed with the CLAM/LC-MS system. A high degree of reproducibility from sample to sample was exhibited using the CLAM-2000. Additionally, this fully automated sample preparation and LC/MS analysis system reduces sample preparation time by humans, increases laboratory efficiency, improves safety while providing high accuracy and reproducibility. A complete sample preparation and analysis solution for drug analysis is possible when the CLAM-2000 is coupled with an LCMS system and Insight software which allows importation of data files into modern LIMS systems for an efficient and productive laboratory workflow.

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Shimadzu's Total Solutions for Clinical Analysis

Chromatography

GC: Chromatographic separation in gas phase for analysis of volatile and semi volatile components has a long history in the clinic, e.g., for quantitative analysis of alcohol in blood.

U/HPLC: Shimadzu offers a wide variety of application-specific systems such as automated sample pretreatment systems for amino acid analysis or on-line sample trapping for quantification of drugs or metabolites.

GC-MS: A hyphenated technique combining the separating power of GC with the detection power of MS. Well known for analysis of drugs of abuse.

LC-MS: Bringing together sensitivity and selectivity, LC-MS is used for the separation, detection and potential identification of compounds in complex mixtures like blood, serum, plasma or urine. Its use is spreading in the clinical field (research and routine) as a replacement for immunoassays.

Spectroscopy

AAS: Used to quantitate concentrations of elements in a vapor.

XRF: Allows analysis of element composition of samples in a wide variety of applications. By analyzing the elemental range from sodium/carbon to uranium its possible to cover the majority of the metallic elements.

ICP-OES: The advantage of plasma compared to other energy sources is the high temperature (10,000 °K), enabling complete atomization of the elements in a sample while minimizing interferences.

UV-NIR: Analysis of metals, ions, colors and molecules. Color reactions, DNA and protein methods are easily applied at low concentrations.

FTIR: Quantification and identification of substances. Infrared spectroscopy can analyze all materials which react with heat.

RF: Quantitative and qualitative analysis of substances. Fluorescence spectroscopy provides low detection limits for the determination of chemo- and bioluminescences and fluorescences from diverse substances. Furthermore, it enables detection of selective DNA or a cocktail of markers in tissue analysis.

Mass Spectrometry

MALDI-TOF: Offers multiple optionsfor rapid profiling of intact proteins extracted from various sources, as well as being a powerful tool for oligomeric DNA analysis

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What Customers are Saying

Hear from four scientists using CLAM-2000 to achieve faster, more accurate results. Follow the links to view the full videos.

Lars Kröner, Labor Dr. Wisplinghoff, finds CLAM-2000 particularly valuable for processing emergency samples without interrupting high-throughput sequences. "We have the CLAM system to make the first step to automated sample preparation for LC-MS/MS analysis. It's basically fire and forget."
https://www.youtube.com/watch?v=dx7iEjX4KuI

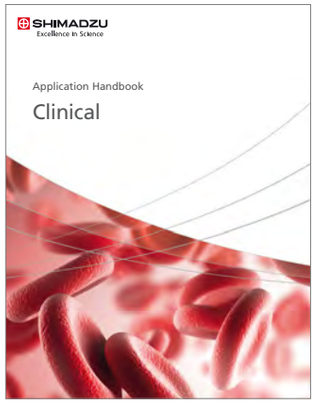
Frank Streit, Head of Clinical Research at University Medical Center Göttingen, highlights the problem of finding trained, experienced personnel to conduct LCMS analyses. Automation by the CLAM-2000 system in his lab allowed non-experts to run the samples, even at weekends.
https://www.youtube.com/watch?v=ge26e7r3X1A

Franck Saint-Marcoux, University Hospital of Limoges, France, describes the future of LC-MS/MS analysis. "You only have to press the button and retrieve the results in a few minutes, just like an immunoassay system," he says.
https://www.youtube.com/watch?v=7-nidowLHys

"I was very impressed with the possibilities the CLAM gave in our laboratory," says Paolo Brambilla, Milano-Bicocca University at Desio Hospital, Italy, who was one of the first adopters of this fully-automated LC-MS/MS platform.
https://www.youtube.com/watch?v=00WSKmcDGp8

Clinical Application Handbook

Collection of application notes and technical reports covering all analytical techniques: GC, LC, GC-MS, LC-MS, Spectroscopy, EDX, ICP-OES, MALDI-TOF MS



Solutions for Clinical Research

Collection of application notes targeted for three important clinical research fields: therapeutic drug monitoring, endocrinology and clinical toxicology.

Full Range of Laboratory Consumables

Shimadzu also offers laboratory consumables such as vials, columns, solid-phase extraction cartridges and much more.

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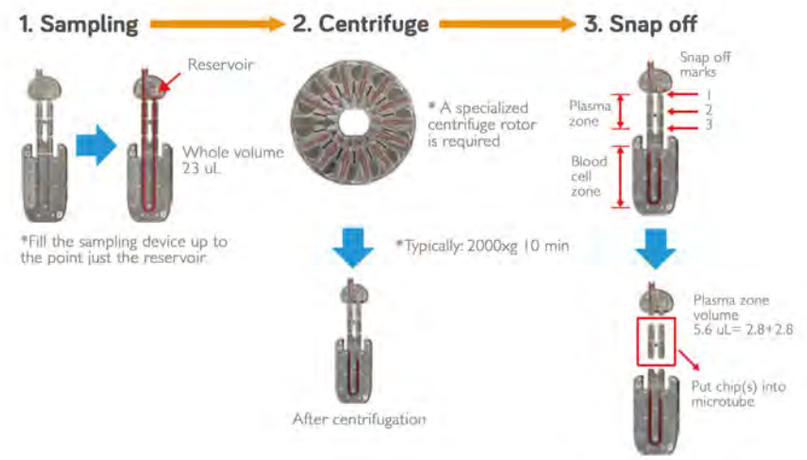
Microsampling Wing & Windmill – an innovative design for plasma collection



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