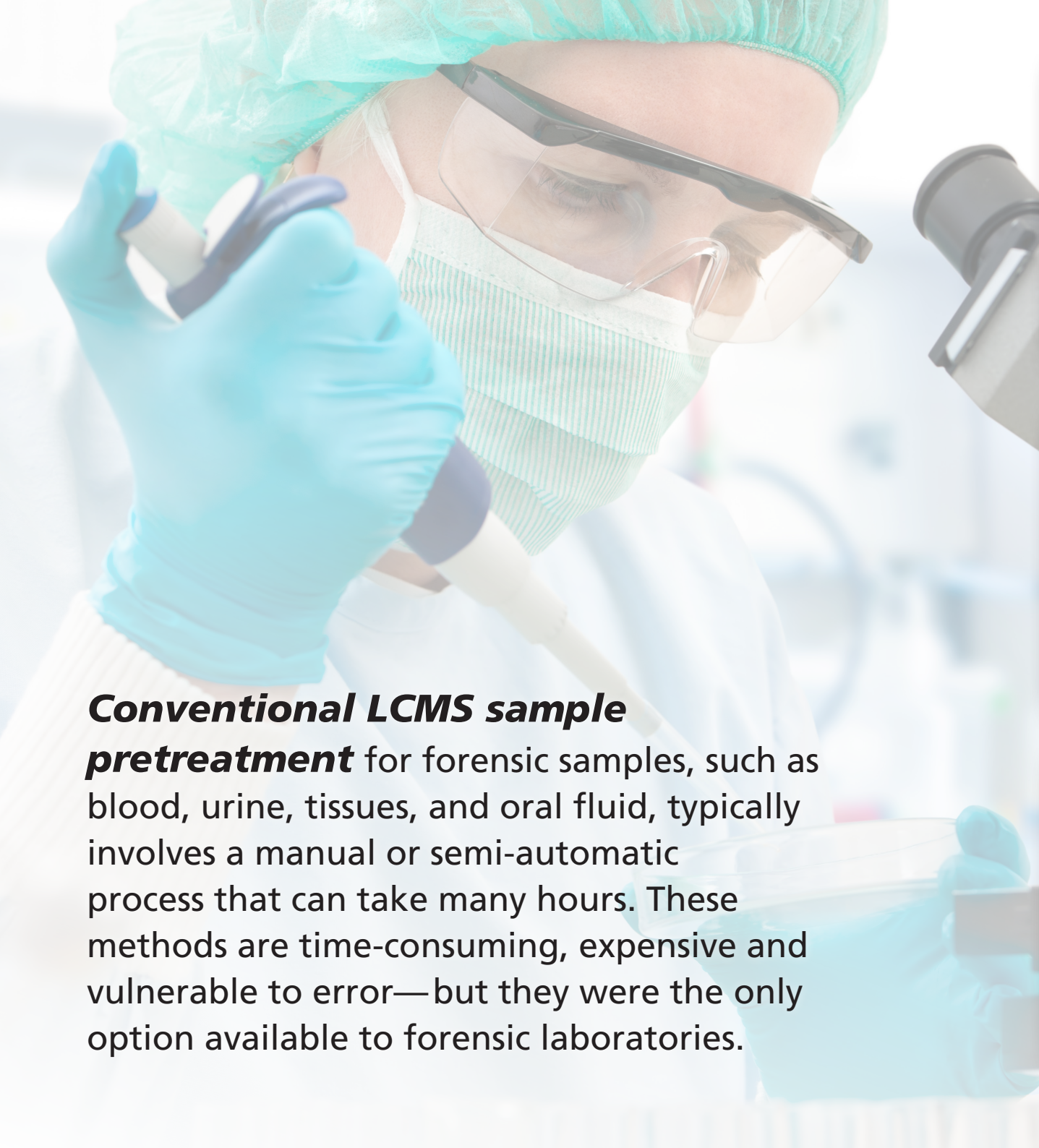


Automating Sample  
Preparation for LCMS  
with Shimadzu's  
**CLAM-2000**  
**SERIES**

**CLAM-2000**  
FULLY AUTOMATED SAMPLE PREPARATION MODULE FOR LCMS



**Conventional LCMS sample pretreatment** for forensic samples, such as blood, urine, tissues, and oral fluid, typically involves a manual or semi-automatic process that can take many hours. These methods are time-consuming, expensive and vulnerable to error—but they were the only option available to forensic laboratories.





The Shimadzu

# ***CLAM-2000 SERIES***

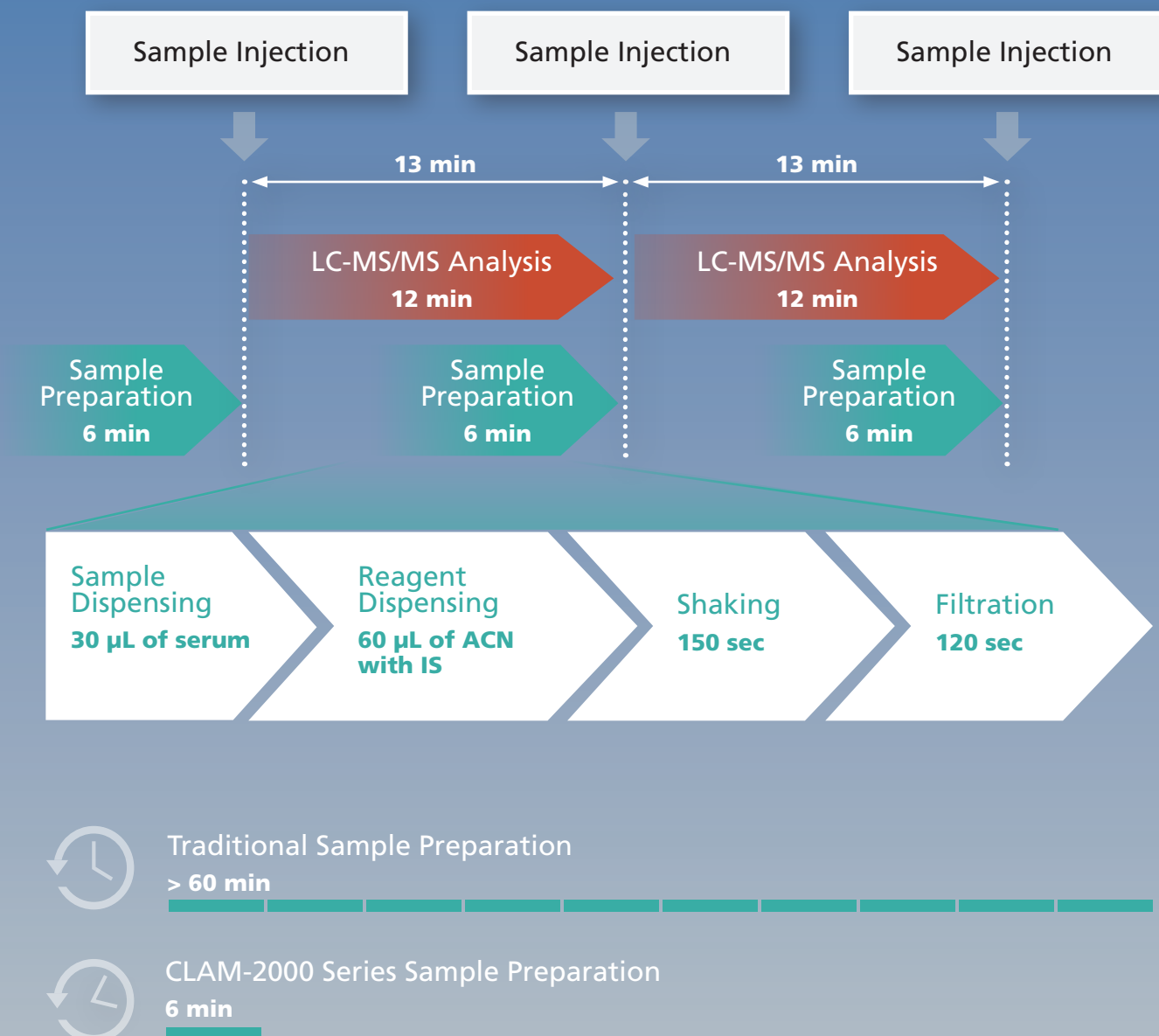
- Fully automates sample preparation
- Minimizes human error
- Increases laboratory efficiency by allowing users to directly place the sample in the system



The CLAM-2000 series provides a **fully automated sample preparation module** that can be integrated with a Shimadzu LCMS analyzer. It was the first system in the world to fully integrate sample prep automation for LCMS workflows, from pretreatment of the sample to chromatographic analysis. Once sample preparation is complete, the CLAM-2000 series delivers the sample to the LC autosampler for injection, removing the need for any human intervention.

The CLAM-2000 series can accept up to **60 samples at a time**, making it the perfect solution for labs of all sizes. An intuitive, easy-to-use interface ensures rapid and reliable operations. Each sample is processed successively in parallel, allowing the system to keep up with current LCMS methods. A fully automated platform helps you speed up analysis, minimize labor costs and greatly reduce human error and contamination.

## Seamless Integration of Sample Preparation and LC-MS/MS Analysis





## ***Fully Automated Sample Preparation & LCMS Analysis of Drugs in Oral Fluid***

Using the CLAM-2000 series, we developed a fast, reliable method for oral fluid analysis. To test the method, we integrated the CLAM-2000 series with a Shimadzu Nexera LC system and a Shimadzu LCMS-8050 triple quadrupole mass spectrometer.

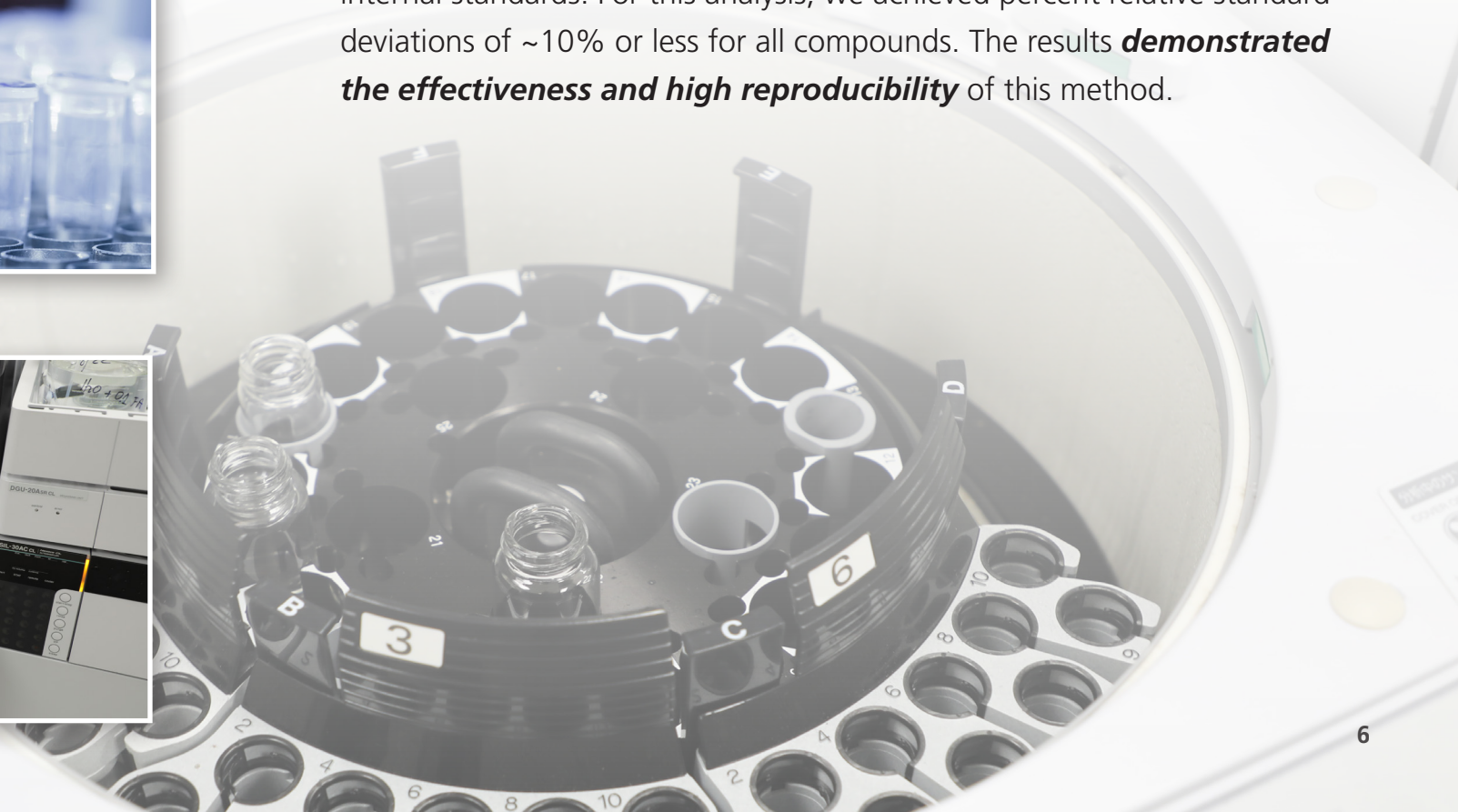




We implemented a linear gradient from 10% to 60% methanol in only seven minutes. All samples were collected with a Quantisal® swab and extracted in diluent. Once the samples were loaded into the carousel, all steps were automatically performed including LCMS analysis. The initial sample preparation steps with LCMS analysis took eleven minutes. ***The sample-to-sample analysis time took seven minutes***, which is equal to the LCMS method time.



The developed method enabled us to perform fully automated sample preparation, LC separation and MS analysis of 122 drugs and deuterated internal standards. For this analysis, we achieved percent relative standard deviations of ~10% or less for all compounds. The results ***demonstrated the effectiveness and high reproducibility*** of this method.





## ***Analysis of Illicit Drugs in Multiple Tissue Matrices Using the CLAM-2000 Series***

The accurate detection of illicit drugs in tissue samples is crucial in forensic science. In many cases, analysis of drugs from various matrices can be vital in determining the cause of death or events that occurred before death.





Fully automated sample preparation modules like the CLAM-2000 series *help improve the efficiency and accuracy* of this type of analysis.

Using the CLAM-2000 series, we developed a method for analyzing drug concentrations in tissue samples. We analyzed a total of **15 analytes** and **4 internal** standards, which are shown in the table to the right. These were run in duplicate in whole blood, liver, brain, spleen and muscle. Linear ranges for most compounds were 10 ng/mL – 1000 ng/mL with a five-point calibration curve in each matrix. The results demonstrated very good linearity for all of the compounds.

Target Compounds and Internal Standards
Morphine-d3
Morphine
Hydromorphone
Codeine
6-AM
Hydrocodone-d3
Hydrocodone
7-Aminoclonazepam
Fentanyl
Buprenorphine
Lorazepam
Clonazepam
Nordiazepam
Alprazolam-d5
Aplrazolam
THC-COOH
THC-d3
Delta-9-THC
THC-OH

A photograph of several clear plastic vials with white caps, containing a yellow liquid. The vials are arranged in a row, with some in the foreground and others in the background. The background is a light blue, textured surface. The vials are sitting on a white, textured surface. In the bottom right corner, there are some colorful, circular objects, possibly part of a laboratory instrument.

## ***Online Sample Pretreatment and LC-MS/MS Analysis for Screening & Quantitation of Illicit Drugs in Urine***

The CLAM-2000 series can be used in a variety of forensic applications. One example is analyzing illicit and prescription drugs in forensic samples such as urine and serum.



Typical automated workflow of urine sample via protein crash and adding internal standard (IS) for LC-MS/MS.

MeOH wetting (conditioning)

Add 20  $\mu$ L of sample (urine)

Add 20  $\mu$ L of IS

Add 40  $\mu$ L of ACN-MeOH

Vortex for 60 seconds

Vacuum filter for 90 seconds

Transferring to LC-MS/MS

We developed a fully automated method of sample pretreatment and quantitation for illicit drugs in human urine. This method was performed using the Shimadzu **CLAM-2000 series** combined with a Shimadzu **LCMS triple quadrupole system**. MRM transitions were monitored for 18 illicit drugs and 14 isotope-labeled internal standards.

The CLAM-2000 series made it possible to automatically prepare and analyze the samples. **Hands-free automation** streamlined the entire process, from reagent mixing and filtration to dispensing solvents and derivatization. An automated batch-run program was also used to perform sample pretreatment and analysis simultaneously. The figure on the left shows the automated workflow we created to test the method.





Method performance was evaluated based on linearity, accuracy, specificity and process efficiency. Reliable quantitation accuracy was obtained for all drugs, except methadone at 20 ng/mL with an accuracy of 130%. The results of the linearity test were  $R^2$  greater than 0.995 for the 18 illicit drugs, with samples ranging from 20 ng/mL to 200 ng/mL in urine. Process efficiency was between 62~122% for all analytes except norbuprenorphine, morphine, MAM and methadone.

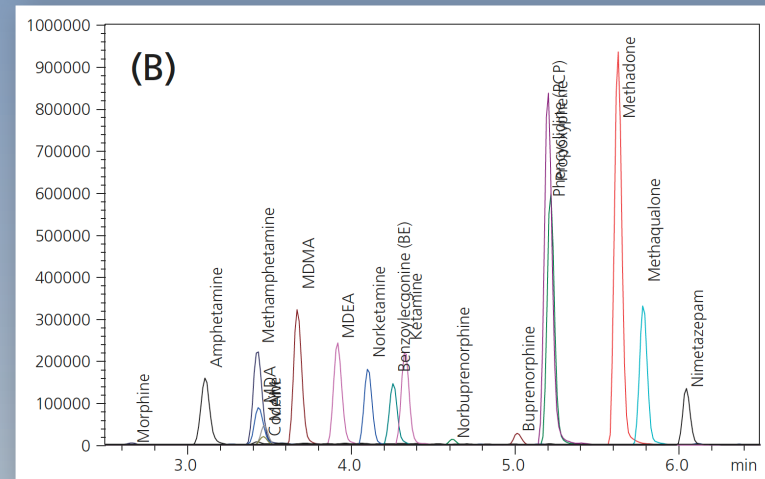
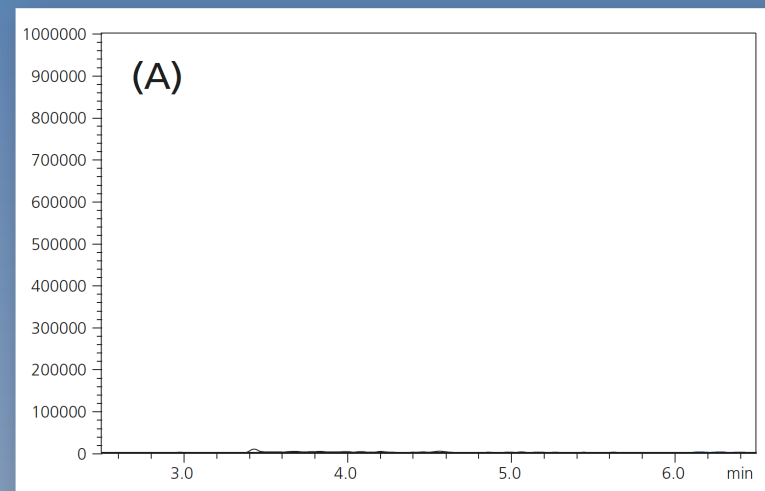
We also found that the method offers high specificity, as indicated by the results in the figure to the right. This figure shows the confirmation criteria for each target, including quantifier MRM peaks and retention times. The CLAM-2000 series delivered the **high-quality results** needed for precise detection of illicit drugs in urine.

Shimadzu's CLAM-2000 series automates and integrates sample preparation, **increases laboratory efficiency** and **prevents sample contamination**—all while providing high accuracy and sensitivity. When paired with an LCMS system, it offers a complete sample preparation and analysis solution for forensic laboratories.



To learn more about how Shimadzu can help you optimize your forensic analysis, visit **[www.InvestigateYourLab.com](http://www.InvestigateYourLab.com)**.

## Total MRM chromatograms of (A) blank urine and (B) spiked urine with eighteen illicit drugs (200 ng/mL).



To learn more about how Shimadzu can help  
you optimize your forensic analysis, visit  
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