SHIMADZU
Excellence in Science

SJ18_0062
ISSN 2188-0484

Shimadzu Journal

VOL. 06 ISSUE 2

Forensics / Toxicology and more...

Director's note

Dear Reader,

It is my great pleasure to unveil Shimadzu Journal Vol.6, Issue 2, which focuses on Forensics/Toxicology. The field of Forensics/Toxicology undergoes continual changes, some related to new regulations and others associated with new or improved analytical techniques being developed. Developing new technology drives Shimadzu's vision, evidenced by both the production of scales and balances for 100 years to the more recent introduction of world-class mass spectrometry platforms that transform laboratory workflows.

Essential to the development and exploration of higher-level technologies and processes is the collaborative research we conduct with our international partners. In this issue, interviews with two distinguished collaborators are featured. One collaborator is Dr. Franck SAINT-MARCOUX, a professor in the Pharmacology and Toxicology department of the Limoges University Hospital (France). He discusses his current research focused on the development of screening procedures using the triple quadrupole LCMS-8060 and fully automated extraction methods carried out by the CLAM-2000 clinical laboratory automation module, directly coupled to a LCMS-8060. He also provides his perspective on the field of clinical and forensic toxicology. You can also read his latest technical report about a fully automated sample preparation procedure to measure drugs of abuse by LC-MS/MS.

The second interview is with Dr. George Hime, an assistant laboratory director at the Miami Dade Medical Examiner's Office. He reflects on some challenges forensic toxicologists face, such as the difficulties in keeping up with the needs of changing sciences and maintaining the ability to detect, measure and identify new substances amid the constant influx of new illegal drugs entering society.

In addition to the special interviews, this issue features the article, Forensic Toxicology in Racing Animals, written by Dr. Paul Wynne, formerly of Shimadzu and now Manager of Monash University's Medicines Manufacturing Innovation Centre in Parkville, Victoria, Australia. Other topics, such as a joint research project between the Shimadzu Innovation Centre (SIC) and National University of Singapore (NUS), are also introduced.

For regular readers, I sincerely appreciate your continuous interest and I am very pleased that it has become one of your favorite journals. Your readership and positive feedback drive us to keep publishing it.

For first-time readers, thank you for taking time to read this issue of Shimadzu Journal. I hope you find the articles in this issue to be of value, and I encourage you to explore previous issues in hopes the content will prove beneficial to both your current work and your future goals. Your kind feedback is highly appreciated.

Yours Sincerely,

Shuzo Maruyama

General Manager, Analytical & Measurement Instruments Division

CONTENTS

Featuring Forensics / Toxicology

Interview with Prof. Franck SAINT-MARCOUX from Limoges University Hospital

Franck SAINT-MARCOUX (PharmD, PhD) holds the position of full Professor of Toxicology and is currently responsible for the Clinical and Forensic Toxicology Unit of the Pharmacology and Toxicology department of the Limoges University Hospital (France). He obtained a Master degree of analytical chemistry, a Master degree of Advanced Studies in Pharmacy and a PhD in Pharmacokinetic Modelling in 2004.
He is author or co-author of over 70 papers in peer-reviewed international journals and delivered a talk in more than 50 international conferences.
Since April 2017, he has worked with the SHIMADZU EU-IC European Innovation Center in the clinical division to develop tools for implementing tandem LCMS in toxicology.
Professor SAINT-MARCOUX, thank you very much for allocating some time for this interview.

To start, can you outline the research you are conducting in general? What is the current state-of-the-art?

We have clinical toxicology and forensic toxicology activities in the lab. This covers numerous fields: accidental exposures to drugs or to toxic compounds, self-poisonings, suspicions of overdose, monitoring of addicts, driving under the influence of drugs (DUID), doping controls… In each situation, it is the responsibility of the toxicologist to ensure an unambiguous identification of xenobiotics involved (when indications are absent), to be able to measure toxicologically high concentrations or to have very low limits of detection, and to provide quantitative results. Most of the time, this has to be possible on a 24/7 basis, with a rapid sample analysis.
However, targets are continuously changing! There are always news drugs for which Therapeutic Drug Monitoring (i.e., individual dose adjustment based on the measurement of the drug in blood) is necessary. There are also continuously new illicit drugs to survey. This explains why we are continuously conducting researches to develop pertinent, modern, practical and efficient analytical methods, mainly using LC-MS or GC-MS.

Can you describe the research you are doing at the European Innovation Center with Shimadzu?

Globally, the research we are doing at the European Innovation Center consists in the development of innovative analytical methods in the field of toxicology. Since the beginning of our collaboration, our efforts have focused on the development of screening procedures using the LCMS-8060 system.
In a first approach, a spectral library has been developed for the Shimadzu LC-MS/MS platforms. It contains over 8000 MRM transitions for 1280 certified reference standards (including 37 deuterated internal standard compounds) with 6084 registered spectra including positive and negative ionisation modes. This library uses product ion spectrum data that can be used in routine library searching and compound verification using reference library match scoring.
One originality of the approach is that we use a novel spectral acquisition method that allows to "reconstruct" a spectrum containing all the specific transitions of a molecule. It is the so-called "MRM spectrum mode". Unlike other previously published approaches where two or three collision energies were applied to all molecules in a method using product ion scanning, we have optimized collision energy for up to 15 transitions from a molecule. This approach makes it possible to

obtain extremely specific and rich spectral information.
Currently, we are conducting research to evaluate the quantitative performances of a screening procedure based on this library. More precisely, we are developing a method for most commonly observed compounds (including antidepressants, anxiolytics, drugs of abuse, analgesics and antipsychotics) and testing its performances at infra-therapeutic, therapeutic and toxic concentrations (quantitative approach). First results are promising and we are close to be able to propose a first procedure.
Meanwhile, we are developing fully automated extraction methods carried out by the programmable liquid handler CLAM-2000, directly coupled to a LCMS-8060 system. A first method has been developed for the determination of 42 amphetamines, cocaine and opiates. These works have been recently submitted for publication.

Why are you interested in this research? What is the goal? Why is it important?

A screening is the first performed analysis when the nature or the presence of a drug is totally unknown, which is often the case in clinical and forensic toxicology. Usually, a screening precedes more specific analyses allowing the quantitation of detected compounds. Procedures that allow both detection and quantitation are awaited.
Additionally, the implementation of automation for all or part of the analysis process eliminates technical errors made by manual preparation and saves time in the laboratory enabling technicians to perform other manual tasks while the system performs the analysis automatically.

How are Shimadzu instruments helping you in your research?

The LCMS-8060 system is a powerful tool with high intrinsic performances. Whatever the application we have developed yet, no loss in sensitivity by acquiring data in MRM Spectrum mode compared to conventional 2 MRM per compound was observed. For example, with the method for the determination of illicit drugs, the dwell time and pause time for data acquisition are typically 3 ms and 1 ms; meaning maximum sample loop time is less than 1 second even at the most intense region of overlapping compound elution. At this time point 220 MRM can be measured simultaneously, but still achieves an average of 20 data points measured across a peak.
The CLAM-2000 is the first system that allows to have procedures where no human intervention is necessary when the primary tube is loaded on board the system. Sample preparation is synchronized with the LC-MS/MS system resulting in no time being lost whilst maintaining the ability to prepare the sample on-line and direct injection immediately after preparation.

What are Shimadzu's strengths compared to other vendors (not limited to instruments)?

Through the European Innovation Center Shimadzu is the first company who proposes us an actual and effective collaboration. This is a win-win and exciting partnership!
All works are under the supervision of both the Department of Pharmacology and Toxicology of the Limoges University Hospital and Shimadzu Corporation (Stephane Moreau, Shimadzu Europa GmbH, Duisbourg, Germany; Alban Huteau, Shimadzu Corporation, Marne-la-Vallée, France). The Mass Spectrometry business Unit (Overseas) Shimadzu Corporation, with Neil Loftus and Alan Barnes, is also greatly involved in these developments. An important point is that we have a PhD student (Tiphaine Robin) who is fully dedicated to the project.

Could you share any requests for Shimadzu for support or technology that would help improve your processes or research, even if these requests may be difficult to fulfill at this moment?

We have multiple projects in mind: such as the development of analytical methods on an upcoming QTOF system, or the development of analytical methods based on microsampling Wing® devices. Unfortunately, we need more people. One potential solution would be sharing a second PhD student with Shimadzu.

Take a look into the future: What will happen in the clinical and forensic fields and how will the change influence analytical instruments, such as Clinical MS, and clinical procedures in 10 years?

In a near future, we will face multiple challenges at the pre-analytical, analytical and post-analytical steps.
Firstly, devices that are less invasive than usual blood samplings, or that allow patients to sample themselves, will become more and more common. This implies that we will more and more face new kinds of samples: dried blood spots (DBS), oral fluids, microvolumes of blood … Nowadays, blood samples are usually not less than 50 to 100 µL. If we anticipate that modern sampling strategies will systematically lead to samples of less 5 µL, you can imagine the gain in sensitivity we will need.
Secondly, more and more drugs will belong to the field of "personalized"or "targeted" medicine. This will imply the development of sophisticated analytical methods as the nano-surface and molecular-orientation limited (nSMOL) that Shimadzu has developed for the measurement of monoclonal antibodies therapeutics.
In the meantime, we have to imagine highly efficient laboratories where the simplicity of operation and the minimization of user involvement in the sample preparation process will be our watchwords. For this, the CLAM-2000 will be very helpful. But, considering my previous remark, the system will have to be flexible enough to treat multiple kinds of samples. I will need to provide it with DBS or with microtubes, and to ask for different kind of extraction procedures.
Very often, the time dedicated to treat/check the data is not considered, while it is a key point. You can't transform any high throughput lab into an efficient lab if you don't have software helping you to rapidly deliver results. Most of the time, I see, in my lab, pretty nice methods with automated extraction procedures and extremely shortened analytical runs, but for which hours and hours are still spent to check the data. Are the calibration curves OK? Are the internal standards areas OK? Are all peaks well integrated? Are the internal quality controls OK? We definitely need tools that automatically answer to these questions and drive us safely, just as new cars can safely drive us on a highway!

CLAM 2000 coupled to LCMS-8060 system: a fully automated sample preparation procedure to measure drugs of abuse by LC-MS/MS

Franck SAINT-MARCOUX, PhD, Full Professor of Toxicology, Clinical and Forensic Toxicology Unit, Pharmacology and Toxicology Department, Limoges University Hospital, France

Abstract

Background:

Measurements of drugs of abuse are needed in multiple contexts within clinical and forensic toxicology (suspicion of overdose, monitoring of addicts, driving under the influence of drugs (DUID), doping control). But, extraction procedures are typically used for LC-MS/MS analysis require manual steps in sample preparation.

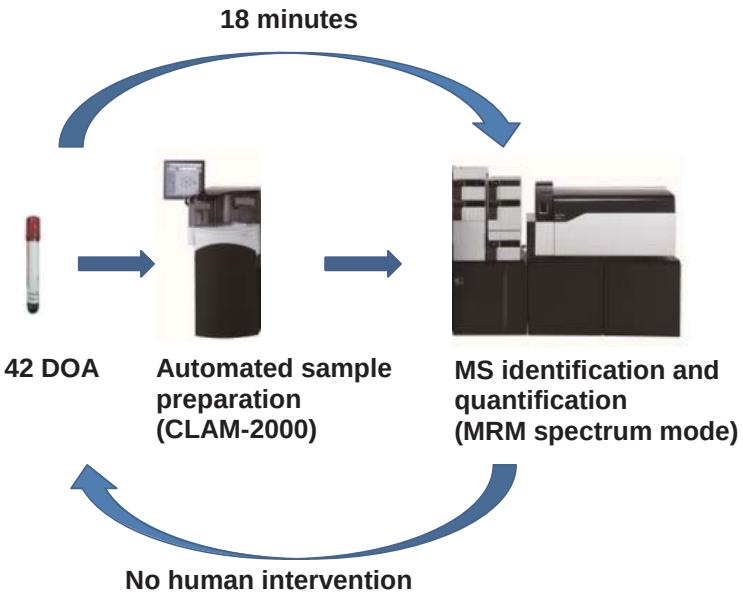
Method:

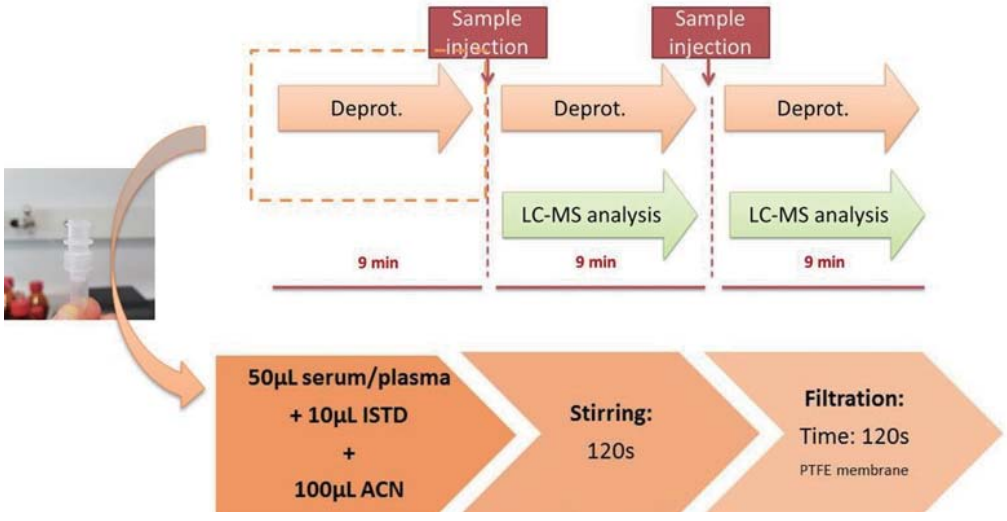
We report a fully automated extraction method carried out by the programmable liquid handler CLAM-2000, directly coupled to LCMS-8060 system for the determination of multiple amphetamines, cocaine derivatives and opioids.

Results:

After placing the sample tube into the system, no further intervention is necessary: automated preparation used 50 µL of blood or plasma with 3 µL of extracted sample injected for analysis. The method was validated according to the requirements of ISO 15189 (repeatability, reproducibility, matrix effects, extraction yields, inter-matrix agreement). Stability experiments showed that calibration curves could provide less than 20% uncertainty for at least 1 month.

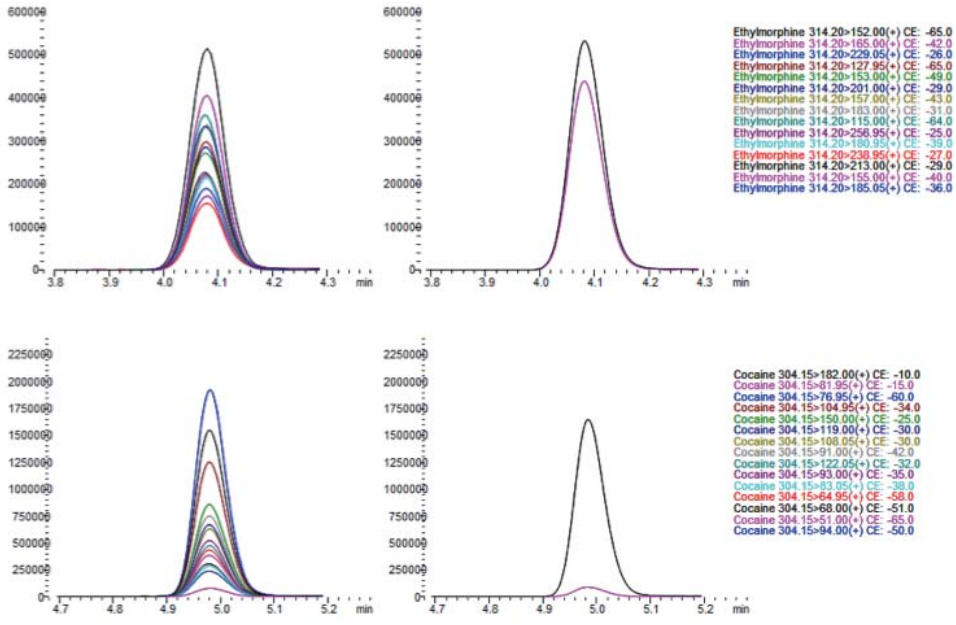
1. Methods and results

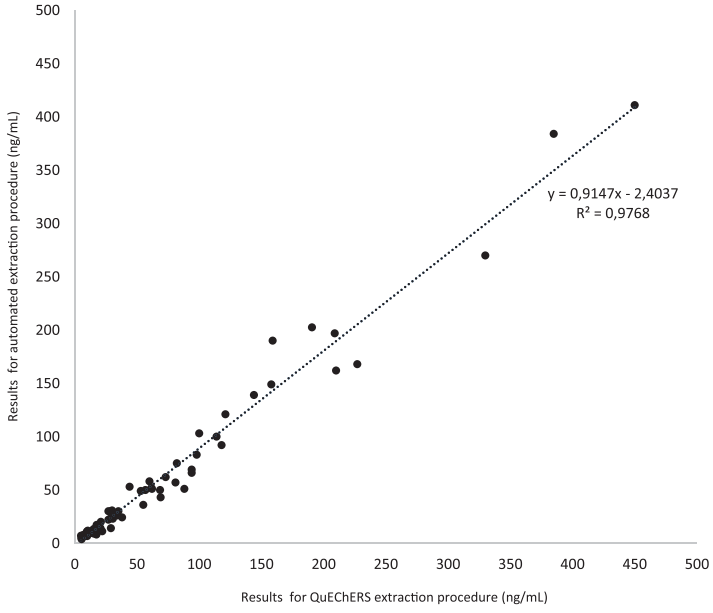




Automated sample preparation was performed in 8 minutes followed by chromatographic separation of the DOA in about 9 min (with an additional 9 min for column wash and equilibration) with retention time from 0.97 min for ecgonine methylester to 7.9 minutes for methadone. About 26 minutes were needed to obtain the first result and then, extraction and separation were performed in parallel with the system producing a result every 18 minutes.

No loss in sensitivity by acquiring data in MRM Spectrum mode compared to conventional 2 MRM per compound. The dwell time and pause time for data acquisition were typically 3 ms and 1 ms meaning maximum sample loop time was less than 1 second even at the most intense region of overlapping compound elution (3.41min). At this time point 220 MRM were measured simultaneously, but still achieved an average of 20 data points measured across a peak.





Biological samples from 43 patients were analysed using both the newly developed procedure and that routinely used in the lab. Nineteen different compounds were detected in these patient samples (opiates, cocaine derivatives and amphetamines) corresponding to a total of 76 data points in a regression analysis. When a compound was positively detected with the validated reference procedure, the sample was also analysed on the new system using the MRM Spectrum mode method.

2. Conclusion

We completed drugs of abuse analysis using a LC-MS/MS coupled to an automated sample preparation system. The results showed the capability of this solution with good accuracy and precision regardless of hydrophilicity-hydrophobicity of the compounds. The accuracy and reproducibility support the feasibility of an integrated and automated system for LC-MS/MS analysis. The simplicity of operation and the minimization of user involvement in the sample preparation process will help transform high throughput laboratories into highly efficient laboratories.

Utilizing the power of the Model 8060 LC/MS/MS to quantify two groups of commonly targeted illegal drugs in postmortem samples

George W. Hime and Joseph Kahl, Toxicology Laboratory, Miami-Dade County Medical Examiner Department, Miami, Florida

INTRODUCTION

George W. Hime has been the manager and assistant director of the Toxicology laboratory at the Miami-Dade Medical Examiner's office (MDME) for over 30 years. Joseph Kahl is a senior supervising toxicologist with the laboratory responsible for the development of quantitative applications.

The toxicology laboratory was established in 1956 as part of the new Miami-Dade Coutny Medical Examiner Department in Miami Florida and consists of a 20,000 square foot facility that has 10 different laboratories, with 12 professional forensic toxicologists and 2 support staff. The laboratory analyzes over 2600 cases per year which entails over 25,000 different tests that consist of screening, confirmation and quantitation of postmortem samples for drugs and other poisons. Forensic toxicology laboratories in the United States that analyze human bodily fluids and tissues have been challenged by the influx of new psychoactive substances (NPS) and designer drugs that have been entering the country over the last 5 years. These substances must be identified and measured with high accuracy to meet the forensic standards of the courts and communities. Often faced with the worst possible matrices such as in postmortem forensic toxicology, the analyst must be able to adapt their methodology to detect very low concentrations of these compounds (< 0.1 ng/mL) while reducing the effect of the background chemical and instrument noise. The analytical instrumentation often is the source of many failures and limitations of any toxicology method. It is important for forensic toxicology laboratories to have the most current and up to date analytical instrumentation so that they are able to accurately measure low levels of these substances in complex matrices. The Miami-Dade Medical Examiner's toxicology laboratory currently has two LCMS-8060 systems. The first was introduced in 2015 and the second in 2018 after receiving a grant from National Institutes of Justice. The addition of the LCMS-8060 into their laboratory has allowed for them to decrease their turnaround time from 65 to 32 days and combine multiple methods into single applications for faster results.

Experimental

Two methods were developed to quantify two common groups of drugs plaguing the MDME laboratory. The first was a group consisting of the most commonly abused drugs found in MDME cases and included: cocaine, cocaethylene, benzoylecgonine, morphine, codeine, 6MAM, and alprazolam. Combining these into a single method would eliminate several tests in the processing of a case and ultimately reduce the turnaround time. The second method included most of the fentanyl analogues currently being detected and considered the cause of death in many of the opioid related deaths in Miami-Dade County over the last 24 months. These include: fentanyl, β-hydroxythiofentanyl, acetyl fentanyl, furanyl fentanyl, carfentanil, butyryl fentanyl, and para-fluoroisobutyryl fentanyl.

This application utilizes the Shimadzu Model 8060 LC/MS/MS Forensic Toxicology Analyzer and can be applied to both the analysis of postmortem whole blood or tissue samples.

System configuration

Liquid chromatograph: Dual Nexera X2 LC-30AD pumps. This is an ultra-high performance liquid chromatographic pump with dual solvent capability per pump. The pump utilizes sapphire micro-plungers (10 uL each) that delivers pulse free solvent flow. It includes an automatic rinse capability and a flow rate capacity of 0.0001 to 5.0 mL with a maximum pressure of 19,000 psi. Included are high efficiency micro reactor mixer to blend small volumes of solvents. The pump setup also includes a high-pressure 2-position 6 port valve for column switching and a 2-position solvent selection valve with a pressure limit of 14,500 psi.

Column oven: Nexera CTO-20AC full size column oven with forced air temperature control from 10°C below ambient to 85°C. Includes an inline membrane degasser that utilizes Amorphous fluoropolymer resin for maintaining a degassed state for mobile phase and autosampler rinse solvents. Includes a 6-position 7 port high pressure switching valve for columns selection.

Autosampler: Nexera SIL-30AC high speed liquid autosampler. Provides high speed (10 sec/10 uL) injections with ultra-low carryover. Optimized for forensic toxicology analysis to reduce carryover by providing 4 solvent rinse capability. The injection needle is placed in the flow path and in conjunction with external as well as internal rinse steps to reduce carryover for injections in the 0.1-50 uL range. Sample vials are cooled using a Peltier system to control temperature. An external rinse pump is utilized to provide additional rinsing of the outside of the sample needle. Maximum pump pressure capacity of 19,000 psi.

Mass spectrometer: Model 8060 triple quadrupole mass spectrometer with DUIS source to accommodate both ESI and APCI type ionization either individually or simultaneously. Capable of a scanning speed or acquisition rate of 30,000 amu/sec (555 channels per sec data collection) at 0.1 amu step size and 5msec polarity switching capability. Incorporates a Qarray pre-quad filtering or ion-guide system which reduces noise and maximizes signal intensity. System includes an electron multiplier with off-axis conversion dynode. Pumping system includes a single triple inlet turbo molecular pump backed up by a 65 m^3/hour roughing pump.

Instrument Operating Parameters

Data Acquisition

Whole blood extracts are prepared and analyzed on the instrument. Calibrators and controls are analyzed concurrently to enable the quantitative measurements of each targeted drug. When available deuterated internal standards are used in the quantitation. The targeted analytes are listed in Table 1. Validation data is displayed in Table 2. The targeted matrix for this analysis is whole blood and tissues. For each targeted drug a quantifying ion and two qualifier ions were collected. Qualitative identification was based on the ratios for both qualifier ions to peak areas to the quantifier ion.

Analyses were performed on a Restek Raptor biphenyl 2.7 micron 50x2.1 mm column with the appropriate guard column. The mobile phase chosen to separate this mixture of drugs was aqueous 0.1% formic acid and acetonitrile programmed using the Shimadzu exponential curve method. The flow rate was 0.6 mL/min. The column oven was retained at 50°C for the duration of the run. The total analysis time to separate all drugs was less than 4 minutes.

Table 1 Concentration ranges and lower limit of detection (LOD) for each targeted drug

	Analyte	Concentration Range	Lower Limit of Detection
1	Morphine	10 – 500 ng/mL	5 ng/mL
2	6-monoacetylmorphine	1.0 – 50 ng/mL	0.5 ng/mL
3	Codeine	10 – 500 ng/mL	5 ng/mL
4	Cocaine	10 – 500 ng/mL	5 ng/mL
5	Cocaethylene	10 – 500 ng/mL	5 ng/mL
6	Benzoylecgonine	0.04 – 2.0 mg/L	0.02 mg/L
7	Alprazolam	10 – 500 ng/mL	5 ng/mL
8	Fentanyl	1.0 – 50 ng/mL	0.5 ng/mL
9	B-Hydroxythiofentanyl	1 – 50 ng/mL	0.5 ng/mL
10	Acetyl Fentanyl	1 – 50 ng/mL	0.5 ng/mL
11	Butyryl Fentanyl	1 – 50 ng/mL	0.5 ng/mL
12	p-Fluoroisobutyryl Fentanyl	1 – 50 ng/mL	0.5 ng/mL
13	Furanyl Fentanyl	1 – 50 ng/mL	0.5 ng/mL
14	Carfentanil	0.2 – 10 ng/mL	0.1 ng/mL

Table 2 Summary of validation data for method across all concentration ranges and analytes

Precision	Inter and intra-day <20%
Dilution range	Up to X10
Bias	-0.3 – 4.4%
Stability of extracts	72 hours under refrigeration
Endogenous matrix effects	Minimal noted
Carryover	None at 2x the highest calibrator
Linear range	0.5 ng/mL – 2.0 mg/L for method 1 1 ng/mL - 50 ng/mL for method 2 0.2 ng/mL – 10 ng/mL for carfentanil in method 2
Within run %CV	1.6% - 4.9%
Between run %CV	1.4% - 3.4%
LOD	0.2 ng/mL
LOQ	0.5 ng/mL
Recovery (blood)	89-92%

Table 3 MRM transitions and collision energy values for each analyte and internal standards

MRM	Analyte	Precursor (m/z)	Quantifier (m/z)	Qualifier 1 (m/z)	Qualifier 2 (m/z)	CE (V)
METHOD 1						
1	Morphine-d_3	289.10	165.20	-	-	37
2	Morphine	286.10	165.20	181.15	201.10	37,36,35
3	Codeine-d_3	303.10	165.10	-	-	42
4	Codeine	300.10	165.20	199.20	181.15	42,30,37
5	6-Monoacetylmorpine-d_3	331.10	165.05	-	-	38
6	6-Monoacetylmorphine	327.95	165.15	211.10	268.10	38,26,24
7	Benzoylecgonine-d_3	292.90	171.10	-	-	35
8	Benzoylecgonine	290.10	168.20	150.20	105.20	35,35,60
9	Cocaine-d_3	307.10	185.20	-	-	35
10	Cocaine	304.10	182.20	150.20	105.20	35,35,20
11	Cocaethylene-d_3	321.00	199.20	-	-	37
12	Cocaethylene	318.00	196.20	150.20	168.20	37,40.40
13	Alprazolam-d5	313.90	286.10	-	-	35
14	Alprazolam	308.90	281.10	274.20	205.15	35,35,25
METHOD 2						
1	B-Hydroxythiofentanyl-d5	364.20	192.25	-	-	25
2	B-Hydroxythiofentanyl	359.20	192.25	285.10	341.05	25,25,25,
3	Acetyl Fentanyl-$^{13}C_6$	329.20	188.20	-	-	35
4	Acetyl Fentanyl	323.20	188.20	105.20	134.10	35,20,30
5	Fentanyl-d5	342.20	188.20	-	-	37
6	Fentanyl	337.20	188.20	105.20	132.20	37,20,34
7	Furanyl Fentanyl-d5	380.20	188.30	-	-	35
8	Furanyl Fentanyl	375.20	188.30	105.00	146.30	35,22,25
9	Carfentanil	395.20	335.20	246.10	279.15	25,25,25
10	Butyryl Fentanyl-d5	356.25	188.20	-	-	37
11	Butyryl Fentanyl	351.25	188.25	105.00	132.00	37,24,30
12	p-Fluoroisobutyrylfentanyl	369.20	188.25	105.00	132.00	37,24,30

Samples

A 0.5 mL aliquot of whole blood is extracted by solid phase (SPE) using a mixed bed column. The extract is evaporated to dryness under dry nitrogen and reconstituted in 50 uL of 0.1% aqueous acetic acid and acetonitrile (90:10) and transferred to septum-capped 2mL auto-sampler vials.

Data Analysis

Calibration curves were linear throughout the concentration ranges specific for each drug with coefficients of variation of greater than 0.99. Controls were prepared and run at two different concentrations for each targeted analyte. Three MRM transitions were monitored for each drug for the quantitative measurements. This included the most

abundant "quant ion" and two additional "qualifier ions". The LabSolutions software provided convenient and intuitive quantitative analysis of data that includes many typical features to improve peak integration, weight calibration levels, and to compare ion ratios as necessary. Color coding of outlying data points enable the identification of problems due to errant calibrator performance or the failure to meet certain criteria.
Identification of targeted analytes was based first on the established ion ratios between the quant ion and the two qualifier ions. Established criteria for ion ratio identification was ±20% across the concentration range. Quantitative analysis was performed using MRM transitions. The selected quant ion peak area for each targeted drug was compared to the selected ion for the appropriate internal standard. A peak area ratio vs concentration linear response curve was established for each analyte. A weighing factor of $1/x^2$ was used in the calculation of the linear curve. Control and sample values were calculated based on the appropriate response curve. Limits of quantitation (LOQ) and limits of detection (LOD) were established for each drug analyte.

Results and Discussion

Over 500 cases (whole blood and tissue) were analyzed utilizing these Shimadzu 8060 Forensic Toxicology Analyzer methods. Concentrations ranged from 0.05 ng/mL to 2.0 mg/L. Sensitivity in conjunction with scan speed enabled the method to collect high numbers of data points for each targeted mass. Peak shape and integration even at the lowest concentrations exceeded expectations. See Figures 1 and 2.

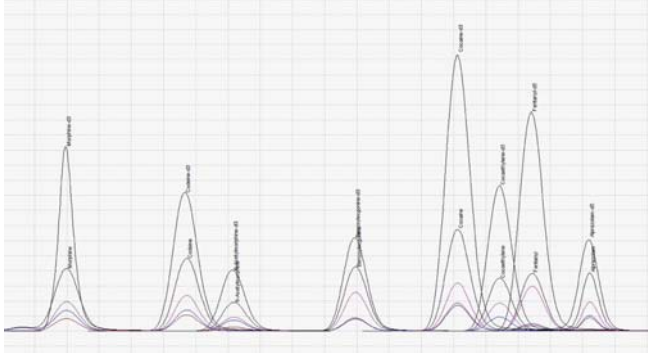


Fig. 1 Collected ion chromatograms for 7 of the targeted drugs in this method, morphine, codeine, 6-monoacetylmorphine, cocaine, cocaethylene, fentanyl, and Benzoylecgonine.

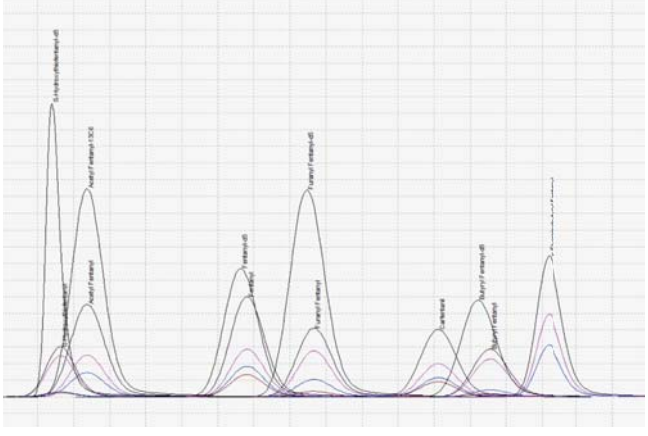


Fig. 2 Collected ion chromatograms for 7 of the targeted drugs in this method, β-hydroxythiofentanyl, acetyl fentanyl, fentanyl, Furanyl fentanyl, carfentanil, Butyryl fentanyl, and p-fluroisobutyryl fentanyl.

In most instances the established LOQ was based on the reporting needs rather than the instrument's capability. LOQ values were optimized using the established method and scanning conditions chosen for the method. Sensitivity can be further optimized to lower the LOD as necessary depending on the need for lower limits of detection. Signal to noise ratios for the method were significantly higher than expected and exceeded what would be necessary to improve the sensitivity by a factor of 10-100x (<0.01 ng/mL).

Conclusion

Applications were developed using the Shimadzu Model 8060 Forensic Toxicology Analyzer to quantify and identify 14 different commonly abused drugs and synthetic opioids in human blood that have been responsible for many of the deaths and criminal intoxications over the last 12 months. The opioids especially have been responsible for acute sudden deaths and is the source for what has become known around the country as the "opioid epidemic".
Limits of quantitation and detection are often established administratively depending on the needs of the laboratory but are most often decided by the targeted drug's toxicity and historical data in death investigations. With the recent fentanyl analogues this information is not readily available. This requires a laboratory to establish these analytical parameters experimentally. It was clear with the development of these two methods that the ability to achieve even more sensitivity was possible with the 8060 while still maintaining very high signal-to-noise. This gives the laboratory more range in their applications especially with drugs such as carfentanil. Achieving parts-per-trillion sensitivities without sacrificing peak integrity or qualitative certainty is a major benefit when challenged by these new illegal drugs.
Combining analytes into single methods enables the reduction in testing procedures utilized in the processing of cases. This procedure reduces the turnaround time and the resulting backlog of cases for the modern postmortem toxicology laboratory. Targeting multiple analytes over a short chromatographic run time can often reduce sensitivity and effect peak integration due to the limited scan speeds of many instruments. This is not a problem with the 8060 system due to the extraordinary scan speed and data collection rate. The results are well-integrated peaks, more than optimal data points collected, high signal to noise, and improvements in method precision and accuracy.
As is often the case in postmortem analysis the role of the matrix in the LCMSMS ion source is of great concern. Ion suppression/enhancement has to be studied and quantified in any new application. However, the design of the source on the 8060 reduces these effects significantly due to the arrangement of the needle and capillary, and the function of the drying gas. A truly unique design that works very well with complex matrices in postmortem analyses.
Due to the nature of many of these drugs and their toxic and lethal levels the instrumental capabilities must be extraordinary. These capabilities must be able to exceed what is normally found in the LCMSMS marketplace with respect to sensitivity and scan speed. Sensitivity to achieve the measurements of extremely low concentrations of the drug in a complex matrix such as whole blood and the scan speed to collect sufficient data points for high signal-to-noise data collection and simultaneous product ion scanning.

Interview with Dr. George Hime from Miami-Dade County Medical Examiner's office

Dr. George Hime, I wanted to thank you for your willingness and time to participate in this interview. First, could you please outline the challenges that the forensic toxicologist faces in today's society?

I believe the biggest challenges facing all forensic toxicologists today is meeting the needs of a changing science with regard to improvements being made on the national level in quality, training, and preparation. With the issuance of the NAS (National Academy of Sciences) report 10 years ago standards of quality are changing rapidly throughout forensic science, but even more so in forensic toxicology. It is these new requirements that are putting more demands on government agencies, funding sources, and the lab personal. It is changing the thinking on how labs are funded and whether to rely on outside reference lab services or the local lab connected to a medical examiner facility for instance. In postmortem forensic toxicology it is my feeling this is a big mistake. Forensic toxicology is done best when there is access to information from investigators and pathologists.

I think another important issue facing forensic toxicologists is maintaining the technological edge and capability to deal with the emerging illicit synthesized drugs. These substances are coming at us so fast. Toxicology labs must remain alert and use all the tools at their disposal to identify these new substances. Being able to detect and measure these unknowns, to understand their chemistry, to be able to interpret their toxicological significance, presents a real challenge. I think what we are seeing today is only the tip of the iceberg. There will be many more to come in the days ahead.

What is your laboratory focusing on to combat these challenges?

Maintaining accreditation and standards for one, and implementing stringent QC procedures is another. Also things such as providing good training programs and maintaining a solid ongoing program of in-house education using workshops, webinars, professional meetings, etc to keep our staff informed on technical trends, instrumentation, and general science concepts in our field. We encourage and support participation, presentations, and publications from everyone in the lab.

Regarding keeping up with the challenges of new emerging drugs; we engage all our staff in the evaluation of case data using a very thorough electronic record keeping system (LIMS/Laboratory Information Management System) that provides them details on investigative information on each case as well as pathology findings. We discuss new tox findings and data such as MS results. Since everyone participates we all learn. No one gets left behind. When we see something new we all learn about it.

Where do you see the role of a post mortem toxicology lab moving to in the future? What is the future of forensic toxicology?

Hopefully the future is bright. As I mentioned before the trend is away from ME (Medical Examiner) labs towards centralized reference labs. This may have some economic advantages but, in my opinion, it is bad for the profession, the medical examiners, and the communities. Considering the history of postmortem forensic toxicology where the toxicologist worked closely with the ME and the investigator to help in determining the cause and manner of death using analytical science, the current trend is away from this collaboration. A big mistake. Unlike the clinical sciences forensic science cannot be conducted effectively in

this manner. The clinical laboratory scientist can work blindly in their testing, the forensic scientist cannot. This field is getting very complicated. Drugs, both pharmaceutical and illegal drugs, poisons, toxins, natural products and industrial toxins are all around us. More than they have ever been. The effects of exposure whether intentional or not cannot be evaluated in a vacuum. If death results an understanding of the total picture must be the goal. Flailing around performing test after test with no understanding of what you are looking for is both a waste of time and money. No matter what kind of capability the lab has this is not productive. I submit the only way postmortem forensic toxicology can be effective is if it is a collaboration of parties.
My hope is that this will be realized and we return to our historical roots, using modern scientific techniques and instrumentation. Only the future knows.

Could you tell us why you chose Shimadzu as your partner when expanding your instrument needs?

The laboratory instrument market is very competitive. But unlike other products we are exposed to as consumers choosing an instrument must be based on a solid understanding of your needs and how you plan to use the instrument you are shopping for. One cannot be blinded by flashy marketing, slick advertisement, and promises of performance that is dubious. When we start the process of purchasing an expensive instrument we define how we will use the instrument to address our needs, what capabilities we are interested in, and then we investigate each manufacturer's offerings. Some manufactures put a new face on old technology. Some re-invent the technology to address deficiencies. But it's always about the details. We found Shimadzu's attention to the details (LC design, source design and function, electronics design, analyzer design, etc) were very good. Efficiency of design in electronics for instance to squeeze the most out of the system was impressive. Also it was clear to us there was widespread confidence and acceptance among other manufacturers in Shimadzu's basic designs such as their LC systems. This told us a lot about reliability and stability of all Shimadzu's instruments. Buying a Shimadzu would not be a waste of money. The customer support and service was the second most important aspect of buying an instrument. If it is good then the instrument is good. That has been my experience after 40 years of buying instrumentation. When the service and support goes down then beware of the instrumentation quality. Shimadzu has delivered on this promise. Our lab has received excellent support for both instrument maintenance and application development. If a company can provide this type of service in this field it should be very successful.

How are these instruments assisting you with the current opioid crisis?

We currently use the 8060 LC/MS/MS system for quantitative measurements of many very potent opioids including fentanyl and its analogues. We needed sensitivity to measure them at relevant concentrations (<0.05 ng/mL) in very messy postmortem samples. The instrument has functioned well at this task. Developing multi-analyte methods was essential because it is more efficient and cost effective.

What are Shimadzu's strengths compared to other vendors (not limited to the instruments)?

Support and training are two big strengths. Some manufacturers seem to be falling behind in this area. A good collaboration between the manufacturer and customer assures the instrument is being properly used and enables the manufacturer to better understand the needs of the customer. I think Shimadzu has excelled at this. Manufacturers must be able to react quickly and use their resources to solve problems. Instrument design seems very well thought out down to the smallest details. Performance specs seem to be more real rather than those proposed by some manufacturers who use software tricks to achieve what they feel is comparable performance.

Finally, could you share any requests that you have with respect to analytical and measuring instruments?

Analytical instruments have gotten more complex over the years. The newer instruments are more software driven than ever before. I think I would like to see more software driven diagnostics capability to aid in troubleshooting. It's an exciting time in analytical instrumentation development. I hope with this advancement comes more user friendliness and continued collaboration between manufacturer and customer. TOF and QTOF instrumentation development will be interesting to watch in the coming years.

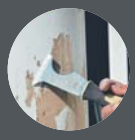
Shimadzu Selection

Shimadzu selected 16 articles for this issue. They derive from application news and technical reports related to forensics and toxicology, and utilize a variety of Instruments we produce. Cutting-edge researches are also included.

Selection 1 Forensics

Screening Techniques in Doping Analysis by GC-MS

This Application News introduces an example of the analysis of a difficult-to-volatilize drug (Screening Method No.2) obtained with the cooperation of MITSUBISHI KAGAKU BIO-CLINICAL LABORATORIES, INC., officially recognized as a WADA testing agency.



Selection 2 Forensics

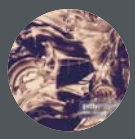
Analysis of Paint Scrapings Using an Infrared Microscope

There are a number of ways of analyzing paint scrapings employing FTIR spectroscopy. Measurement can be carried out by crushing the collected paint scraping and employing the KBr pallet method of diffuse reflection method. Introduced here is an example of measurement of paint scrapings from a timber surface.

Selection 3 Food Contaminant

Contaminant Analysis in Food Manufacturing Process by EDX and FTIR

EDX and FTIR are widely used for analysis of foreign contaminant matter, but recently, these instruments are increasingly being utilized in tandem to conduct contaminant analysis. Intoduced here is an example of actual analysis if various types of foreign matter entered during the food manufacturing process.



Selection 4 Chemical

Analysis of Thermally Degraded Plastics Using Thermally Degraded Plastics Library

In this article, we introduce an example of the changes to the infrared spectrum of a plastic degraded by heat, and a sample search using a library containing data created by changing the heating temperature and time beforehand.

Selection 5 Pharmaceutical

Analysis by ICP Atomic Emission Spectrometry in Accordance with the ICH Q3D Guideline for Elemental Impurities Using ICPE-9820

We conducted 24 elements according to the ICH Q3D guidelines using the Shimadzu ICPE-9820 multi-type ICP atomic emission spectrometer. The ICPE-9820 offers simultaneous all element analysis with high sensitivity and high precision, while delivering high throughput.

Selection 6 Toxicology

Direct Determination of Pb in Whole Blood by Graphite Furnace Atomic Absorption Spectrophotmetry (GF-AAS)

This application news will report on the analysis of Pb in whole blood using the Shimadzu AA-7000G (Graphite Furnace Atomizer) and the Platform-type Graphite Tube. The method presented is a quick way to determine Pb in whole blood as the sample was mixed with a matrix modifier solution prior to GF-AAS analysis.

Selection 7 Toxicology

EDXRF Analysis of Cd, Hg, and Pb in Blood

There is a need nowadays for the rapid estimation of the various type of poinsons in medicine that have combined with metals in order to be able to give emergency treatment through the administering of an antidote.

Selection 8 Forensics

Dual Channel Blood Alcohol Content (BAC) Analysis

Using the Shimadzu GC-2010 Plus and HS-10 headspace sampler, this application note demonstrates that by moderately increasing column temperature to 50°C and using the optimal flow rate for capillary columns, BAC (Blood Alcohol Content) analysis can be completed in less than 3.5 min without sacrificing peak shapes.

Selection 9 Pharmaceutical

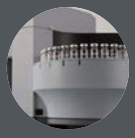
Quantitative Analysis of Cannabinoids using the LCMS-2020 Single Quad MS

Evaluation and quantitation of a variety of cannabinoids on an LCMS-2020 single quadrupole mass spectrometer.

Selection 10 Forensics

Detecting New Designer Cannabinoids in Herbal Incense Using LC-MS/MS with Fast Precursor Ion Scanning

We developed LC-MS-MS methods that utilize extremely fast precursor ion scanning for detection of designer cannabinoids in herbal incense products. The urine of human subjects who reported synthetic cannabinoid exposure was also analyzed using a newly developed high sensitivity triple quadrupole mass spectrometer from Shimadzu, the LCMS-8040.



Selection 11 Forensics

Analysis of Blood Alcohol by Headspace with Simultaneous GC-FID and MS Detection

This application note describes BAC(Blood Alcohol Content) analysis using a GC-FID in parallel with a mass spectrometer (MS) for positive compound identification.

Selection 12 Forensics

GCMS-QP2010 Series Determination of Drugs of Abuse in Oral Fluids

The methods in this booklet are a compilation of work collected from working forensics laboratories. Each of these labs is a Shimadzu customer and all of the data was generated using the GCMS-QP2010 series of instruments.

Selection 13 Toxicology

Analysis of Toxicological Substances in Whole Blood Using Smart Forensic Database (1)

This article introduces an example of applying Smart Forensic Database to the analysis of toxicological substances in a whole-blood sample.

Selection 14 Toxicology

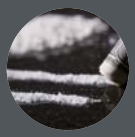
Analysis of Toxicological Substances in Whole Blood Using Smart Forensic Database (2)

This article introduces an example of using a simultaneous Scan/MRM measurement method created from the Smart Forensic Database to measure a whole-blood sample, and then applying the GC/MS Forensic Toxicology Database to the scan data obtained.

Selection 15 Toxicology

Semi-Quantitation of Toxicological Substances in Whole Blood Using the Quick-DB Forensic

This application data sheet introduces an evaluation of the semi-quantitative accuracy by calculating quantitative results for a pretreated sample of whole blood with drugs added, using calibration curves registered in Quick-DB Forensic.



Selection 16 Forensics

Analysis and Quantitation of Cocaine on Currency Using GC-MS/MS

This application note describes a method for extraction, identification, and quantitation of cocaine on paper money from nine different geographical areas around the globe, including five samples from the United States, using the Shimadzu GCMS-TQ8040 triple quadrupole mass spectrometer and the Multiple Reaction Monitoring (MRM) monitoring mode.

Forensic Toxicology in Racing Animals

Paul Wynne, PhD, Manager, Medicines Manufacturing Innovation Centre, Monash University, Victoria, Australia

A brief introduction

Amongst toxicologists, there has always been some debate over whether or not the detection of drugs and other residues in samples collected from racing animals and human athletes is a form of forensic toxicology or falls into some other category. The closest parallel is probably policing work where the legal principle of reverse onus applies. After many years working in the sector and then more recently working alongside the industry developing equipment and techniques, it has become clear that such residue testing is well categorised as forensic in nature. There is often a compelling detective story that is associated with a sample and the chemical analysis forms a part of the brief of evidence that defines the case with which it is linked.

The use of drugs in sport is associated with changes in performance that allow unfair advantage or, less commonly, disadvantage. In those cases where the animal has a jockey, rider or driver then there is an expectation that performance altering substances might be given to make the animal run faster or further because the human could act to achieve the reverse effect. In events that rely on calm and controlled behaviour, advantage might be achieved by the use of anti-anxiolytics or sedatives that support task-specific activity and supress distraction. Where the animal races without a passenger, for example in greyhound or pigeon racing, there may also be unfair advantage gained across the field by using substances to slow an individual animal and so lessen its chances of winning.

Experience in the field suggests that the detection of drug residues in pre- and post-event samples falls to less than one percent of all samples tested in a regulated environment. Further, of the samples that test positive, many show the presence of therapeutic substances used to recover from injury and so might suggest (but does not prove) mistiming of the withdrawal of medication rather than more serious intent.

I do not intend to discuss the application of the rules of racing in international jurisdictions or debate the merits or otherwise of reverse onus, zero tolerance, thresholds or screening limits in this article. These matters are, rightly, for the industry to debate and act upon. For the scientific community, what makes the Forensic Toxicology of Racing compelling is the clever application of measurement techniques to detect low levels of residual substance that often are extensively metabolised and present in difficult matrices. With this in mind, when I was asked to contribute to this issue of the Shimadzu Journal, I understood that I had the opportunity to reflect not just on the changes in testing approaches that the industry has experienced with time but more importantly to consider how technological innovations from instrument vendors such as Shimadzu have changed the way the analyst thinks about the analysis of their samples.

An interface between the animal and a report

As with all forensic toxicological analysis, the racing chemist is challenged to translate the events and variables represented by a single biological specimen, collected under very specific circumstances, into a report that is numerical and absolute in its conclusions. Distillation of this kind is not simple and the laboratory's priority is to report on the basis of methods that allow unequivocal identification of identity. Substances that are known-unknowns become the target of screening and confirmation while unknown-unknowns remain the target of more open testing methods and ongoing research.

Racing jurisdictions, regardless of their approach to regulation, work on the basis that identification of a substance or drug residue in a sample will meet reverse onus criteria. Simply put, the laboratory must make a strong case to justify their reporting of the presence, and in many cases the concentration, of a substance in a sample. The report is thereafter considered as prima facie evidence that an offence under the rules of competition has been committed. The reverse onus approach to reporting is common to many regulatory fields and requires the defendant (for example a horse trainer) to establish that the laboratory was wrong in its analysis or report or that there are mitigating circumstances that explain the presence of a prohibited substance in the sample.

The scientific approach to analysis must use the informing power of the data that is generated to satisfy the certainty requirements of the analyst. In most cases, a suitable standard of proof is provided by the combination of specific chemical behaviour of the substance (for example, its extraction, derivatisation and chromatographic characteristics) and the response it elicits from a specific detector (for example its mass spectrometric behaviour).

It is not surprising with this need for unequivocal identification of substances that the work of the racing analyst has evolved in parallel to the development of chromatography and mass spectrometry techniques. An important part of the discipline is accepting that we are not 'there' yet in providing the ideal service and that change will continue to influence both the substances and practices that are detectable as well as the methods that we use to detect them.

Data quality and informing power

Informing power in chemical analysis has been described in many ways but is generally multiplicative across complementary techniques. That is, the combined informing power of an extraction chemistry, a chromatographic retention time and a mass spectrum is inherently more informative than the sum of the information contained in each part. In a chemico-legal context, high quality data that includes orthogonal or reinforcing techniques is preferred.

At the same time, laboratories face pressure to increase the throughput of both samples and the number of analytes that are reported. Inevitably, such pressure drives method unification strategies within the laboratory and decreases the information that flows from specificity in sample preparation, derivatisation and chromatography. A logical consequence of this trend is to expect that the detector is capable of making up the short fall in unique data that was previously expected of a more specific method.

By way of example, my career in Racing Chemistry began at a time when GC-MS was emerging as a screening rather than confirmatory technique and LCMS was available only as a specialised technique in a few laboratories. Innovation was supported by new approaches to sample preparation and SPE began to supplant liquid-liquid extraction because it was cost-effective but also significantly more selective and therefore more informing.

Our group was enthusiastic in embracing mixed mode SPE as a way of improving the specificity and selectivity of analysis[1]. The approach dealt with the complexity of the equine urine and time consuming aspects of manual sample handling by extracting one portion of hydrolysed urine and manipulating the retentive chemistry of SPE devices to produce a number of sample streams (Fig. 1).

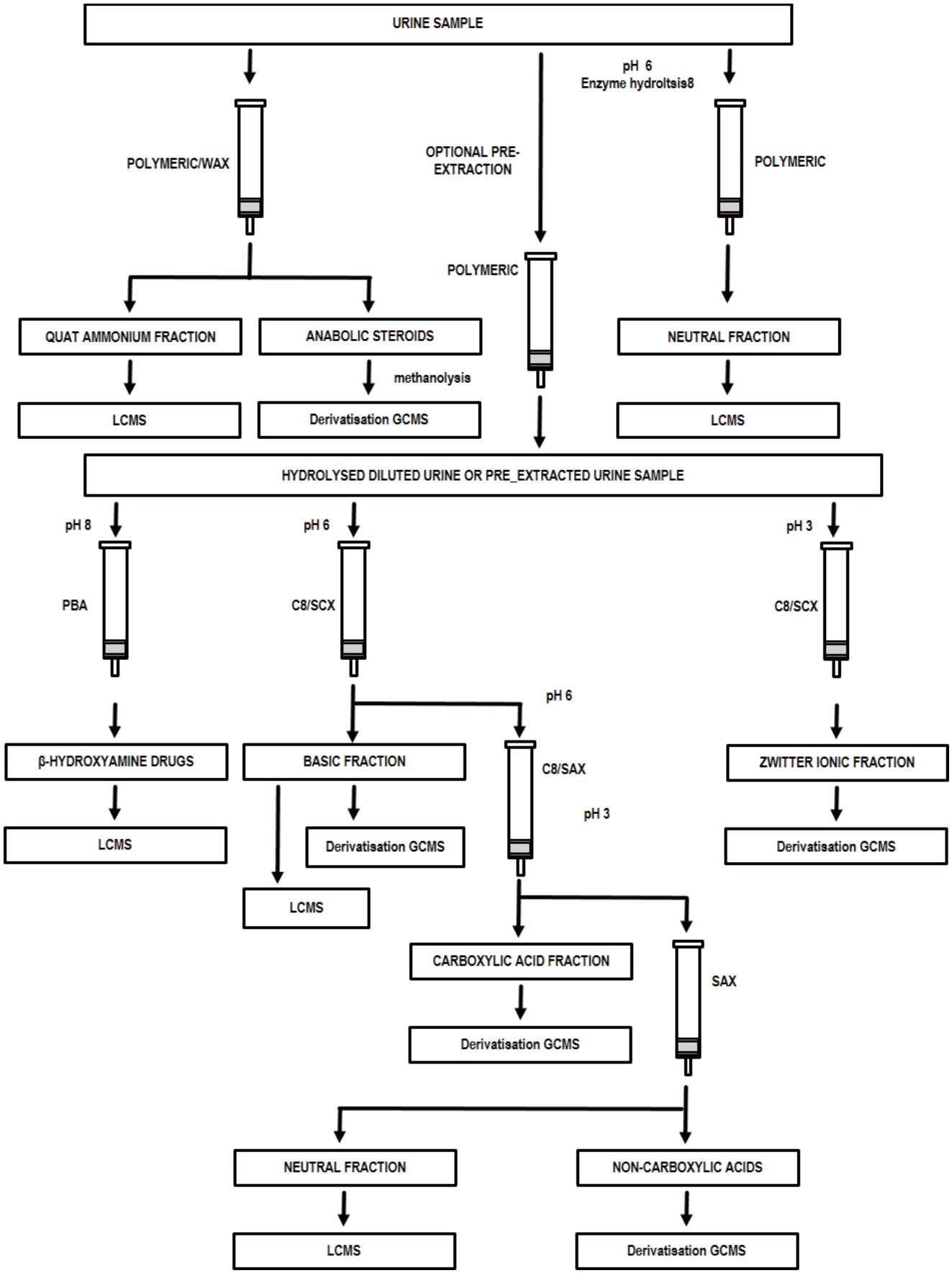


Fig. 1 Sample matrix complexity and the expansion in number and class of analytes for which testing was sought led to the development of sample preparation methods that were suitable for the existing instrument platforms and influenced the data flow from an analysis.

The basis of this approach was that many short analyses that targeted specific groups of analytes provided better workflows, higher quality data and reduced instrument maintenance than universal testing methods. An inevitable characteristic of the shift away from simpler methods was a change in the way the racing chemist assessed the non-specific component of the sample. Where a method involved improved specificity for functional chemistry (for example urinary bases) but chromatography and mass spectrometry was non-targeted, the opportunity existed for profiling of the population in an information environment that predated metabolomics as we know it now. Where the method was targeted, processing efficiency was increased but profiling (non-targeted) opportunities were lost.
Examples of unusual substances been detected in samples on the basis of pattern recognition were common in the literature of the time. In a sense, the methods of analysis filled the requirements of Systematic Toxicological Analysis but the data analysis was still influenced by the subjective experience of the analyst. From my own experience, the use of extraction selectivity and non-target screening let to many cases of new compounds been detected and two notable examples of substance abuse.
The detection of a hydroxylated metabolite of 2-amino-4-methylpyridine[2], was enabled by a shift to mixed mode SPE in combination with non-targeted GCMS screening and an alert analyst who questioned why the unidentified early-eluting peak in several related samples had not been seen in samples previously tested with the same method. The analysis that followed would be classified as research by the laboratory but also included co-operation between agencies to gather disparate pieces of information into a sensible timeline of events. The analyst was expected to be dogged in their pursuit of a completed analysis and so was born a detective story. The parent compound has significant physiological activity and circumstances surrounding the case supported the charge that it had been abused. It is unlikely that the compound would have been detected in a targeted testing regime and illustrates that understanding the routine population provides intelligence that can inform decision making.
The discovery of bags of the parent compound hidden in bushland near the stable in question provided physical evidence in support of the analysis carried out on the urine and linked the material to a supply chain (Fig. 2).

A second example from the same method was the detection of elevated levels of 3-methoxytyramine in a number of samples[3]. The substance detected is an endogenous metabolite of the dopamine cascade and was again noted on the basis that it was present at concentrations considered extraordinary by the analysts working through non-targeted screening data (Fig. 3).
The samples were eventually determined to be irregular and an International threshold for urinary 3-methoxytyramine was subsequently introduced to the rules of horseracing. Examples of threshold substances had been established in racing chemistry and include hydrocortisone, testosterone and bicarbonate. With other substances, there was a direct availability of the threshold substances for administration and intelligence within the industry to suggest that they could be abused. In contrast, while 3-methoxytyramine is only one step removed from dopamine it is two or three metabolic steps removed from the likely precursors substances (L-tyrosine or L-DOPA). The case is relevant to my discussion in that 3-methoxytyramine was not predicted as a screening target (although perhaps it should have been) and was discovered during non-targeted GCMS screening. For my own group, it was also important in that it strengthened the internal case to consider how metabolomics might impact on future drug screening strategies. Subsequently racing chemists have developed a better understanding of the dopaminergic system and have questioned the relevance of the urinary concentration as an indicator of physiological effect following oral precursor supplementation (a bioavailability argument) but also investigated metabolomic shunting or masking through the use of catechol-O-methyltransferase inhibitors[4].

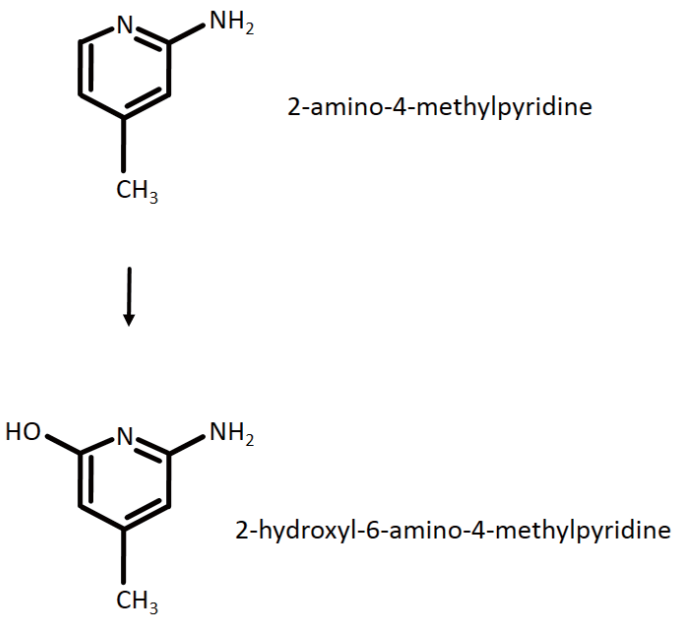
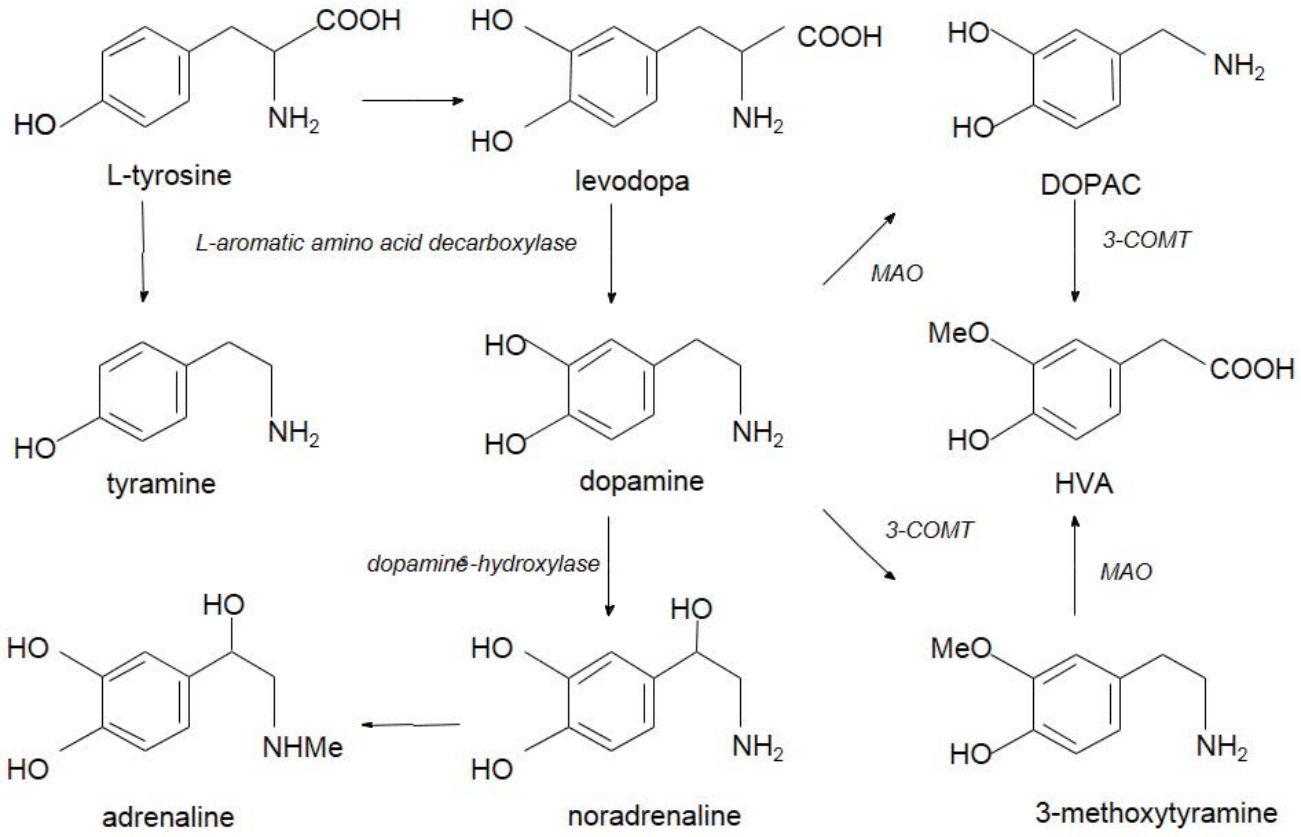


Fig. 2 The detection of 6-amino-2-hydroxy-4-methylpyridine in race-horse urine based on non-target screening by GCMS was linked to the administration of 2-amino-4-methylpyridine. The parent compound was found in prepared doses hidden near the property where the horses were stabled and provided physical evidence that linked the material to a supplier.



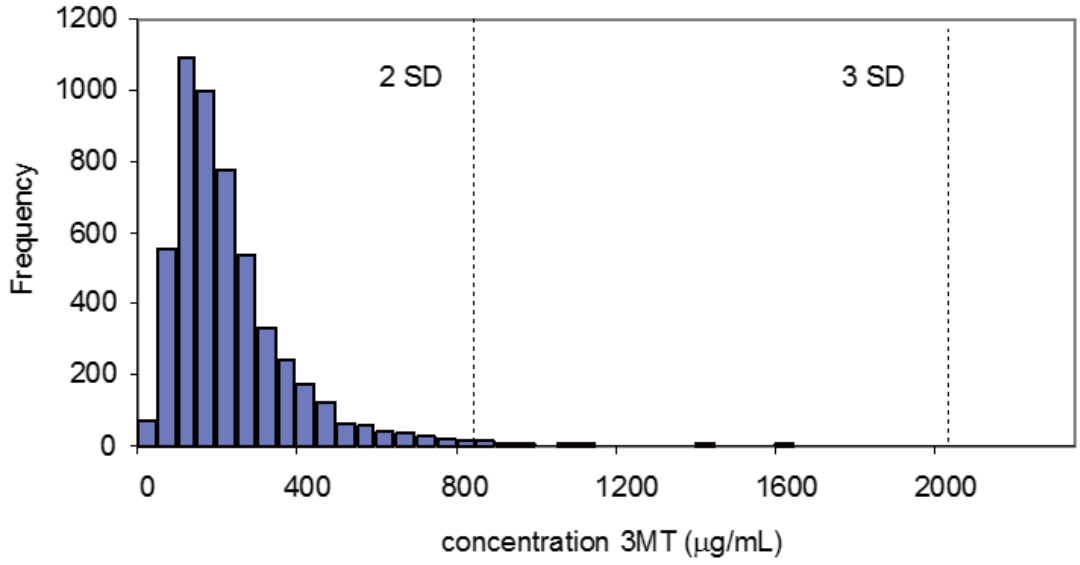


Fig. 3 The detection of elevated urinary concentrations of 3-methoxytyramine was originally the result of pattern recognition within a population. The compound is a terminal metabolite of dopamine and studying its concentration in urine let to the development of a regulatory threshold concentration for the substance has made this endogenous compound a target analyte.

It is reasonable to wonder why I have highlighted these two examples from the many that were detected by the same method over years of testing. They are illustrative of the dilemma that drug screening programmes face insofar as the need to manage workload with target screening (known-unknowns) with the concomitant risk that non-target substances (unknown-unknowns) will be missed. I am more concerned that the loss of the so-called open-ended screens also result in a loss of the population intelligence that such methods have the power to provide. One might posit that the ideal solution would be detector instrumentation that allowed both targeted and non-targeted screening to run simultaneously. We would expect that such a method would not compromise detection of either the target panel or non-target compounds but know that such an expectation can only be supported by instrument design.

In the case of GCMS instruments, data processing has provided an excellent opportunity for extraction of targeted information from a full-scan data set. The extraction of a selected ion chromatogram is a simple way of increasing the specificity of a view from a larger data set. However, the sensitive detection of low level substances and the improved informing power of MSMS spectra have influenced many laboratories to acquire GCMSMS instruments even though there is an overall trend away from GCMS toward LCMS techniques.

Gas chromatography maintains an advantage over liquid chromatography in that the former technique offers selectivity towards fine functional structure, exceptional peak capacity and an orthogonality in chemistry to liquid-liquid extraction or SPE methods[5]. When combined with mass spectrometry, the technique provides for the detection of difficult to ionise compounds and access to the extensive libraries of electron-impact mass spectra that are available both commercially and within organisations.

In many cases, GCMS continues as the method of choice for the targeted analysis of anabolic steroid metabolites that are not amenable to LCMS analysis[6]. Interestingly, the methods are rarely used for non-target testing because the derivatisation reagents do not lend themselves to an easy to interpret chromatogram but that opportunity is there to do so.

I am particularly interested in the use of GCMSMS (Fig. 4) by colleagues for the screening of a range of derivatised basic and acidic substances in which targeted screening by EI-MSMS or EI-MS was combined with the simultaneous non-targeted screening by EI-MS[7]. The study was initially concerned that using EI as a method for generating precursor ions would reduce the method sensitivity for MSMS because the analyte's ion population would be spread across many fragments rather than a single abundant protonated molecular ion expected if using chemical ionisation. In practice, the work found that for most drugs EI afforded not only equally or more abundant ions than CI but also the choice of more than one 'high value' precursor ions for MSMS purposes.

A method was run in EI mode on a UFMS capable instrument and therefore allowed not only targeted MSMS but also a background scan event that preserved the non-target screening capacity of the method and its profiling information. An added benefit of the method was that a MRM response that suggested the presence of a prohibited substance on the basis of signal to noise could be checked against the EI-scan spectrum acquired in the non-target data channel. In terms of informing power, EI-MS continues to be the benchmark for many compounds but becomes even more so when it can be combined as a high-speed data channel with EI-MSMS experiments.

The method is an elegant way to meet the emerging need for through-put, sensitivity and selectivity while also capturing non-target screening data and profiling information that can be monitored for changing trends, metabolomic perturbations and other effects. Achieving the desired outcome without loss of sensitivity required innovation by the instrument manufacturer to deliver sufficient speed (Ultrafast Mass Spectrometry) to perform all the tasks desired without loss of sensitivity, mass axis displacement or missed data.

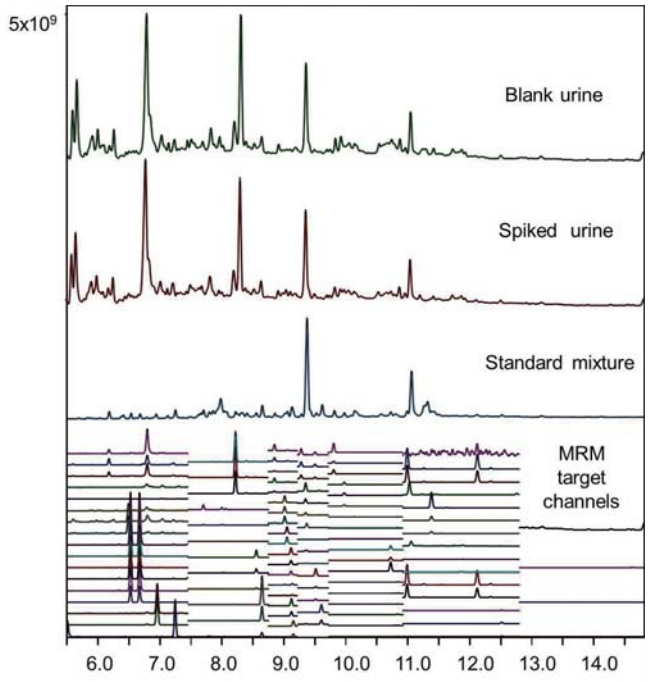


Fig. 4 A Shimadzu GCMS-8040 allows the simultaneous acquisition of non-targeted EI-GCMS data that can be searched against commercial and in-house EI libraries as well as targeted MRM data for sensitive detection of analytes.

I have touched on methods that have evolved to use sample preparation (speciation) to feed chromatographic instrumentation and the importance of both targeted and non-targeted screening methods to facilitate an effective drug testing programme. The era of testing that I describe was elegant in its treatment of analyte chemistry but was also developed by a generation of exponents, myself included, who predicated their method design on a strategic reliance on gas chromatography.

Winners and losers in the LCMS revolution

Over the past generation, the most significant change to the reporting of prohibited substances detected in biological samples has been the widespread application of LCMS. The technique has allowed racing chemists to monitor increasingly functionalised small molecule targets that were either not suitable targets for GC inlet or at least required extensive modification. Some examples that emphasise small molecules and functional groups that impart polarity are illustrated in Fig. 5. The examples are intended to be thought provoking rather than an exhaustive list.

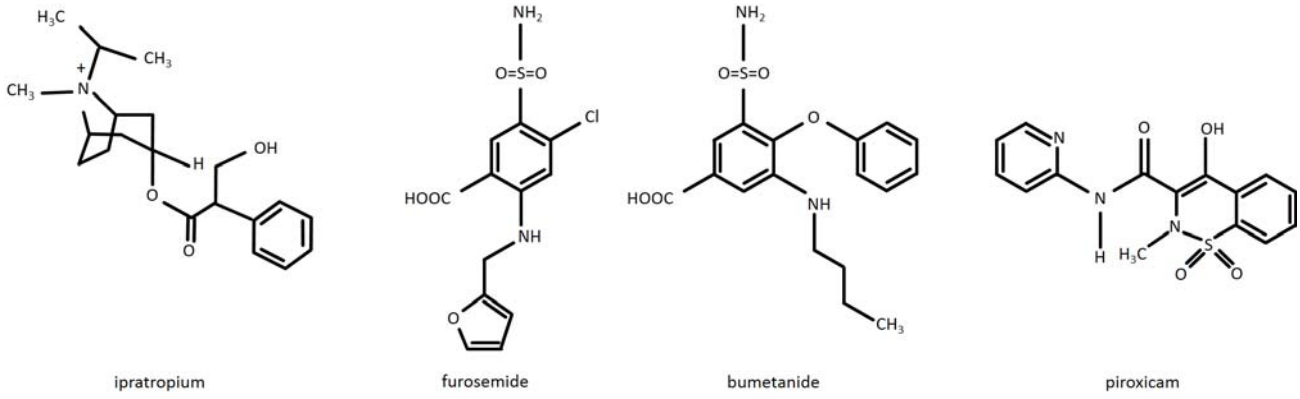


Fig. 5 Examples of small molecules that are more easily analysed by LCMS than alternative techniques include the quaternary ammonium compounds (e.g. ipratropium), diuretics containing sulphonamide and related groups (e.g. furosemide and bumetanide) and the COX inhibitors (e.g. piroxicam).

With an increasing target compound list, the racing chemist has been challenged to look for sample preparation techniques that are able to offer some matrix component removal and a degree of concentration while also recovering diverse compound chemistries into a single fraction. The fraction or extract is usually intended for screening by a reversed-phase LCMS techniques that is designed around effective ionisation of as many compounds as possible. Such approaches are currently the only way to achieve cost and time effective coverage of the many different compound types that are available for abuse or of interest to the industry.

The trend defines one of the main requirements of modern drug testing programmes in that they must offer extraordinary capacity to detect analytes at low concentrations in complex biological samples such as horse or greyhound urine. My own observation of the current approach to screening is that the nuanced elegance of sample preparation that underpinned the methods of previous generations of testing is being lost in the process. As the methods have chased recovery of increasingly polar analytes and metabolites, the management of the polar matrix has become a less important consideration in method design.

The consequence for the instrument designer and for the racing chemist is that there is no longer room in the methods for non-target methods and that there must be an expectation that the mass spectrometer is able to offer sufficiently high informing power to overcome the loss of orthogonality (over previous approaches) that comes from an essentially reversed-phase sample preparation step being combined with a reversed-phase chromatographic separation.

Two approaches to analysis have emerged. One has made use of the exquisite mass filtering of HRAM-MS to separate target compounds from the background while the other has used the inherent sensitivity and speed of the triple quadrupole mass filter design for targeted MSMS analysis. Neither is perfect but both offer distinct advantages to screening programmes and many laboratories now employ both techniques in concert. HRAM-MS applied to target compounds with a data set that can be target-mined for new compounds while q-HRAM-MS is used with the advantages of target screening that includes more precise mass filtering to give higher informing power. In contrast, TQ-MSMS is applied to high throughput multi-target analysis and is increasingly able to accommodate increased informing power through high-speed scanning, polarity switching and the acquisition of multiple iterations of MS^2 data across a peak - a technique I have referred to as nMS^2 in recognition of its contribution to identification[8,9].

In all cases, I am reminded that we train students to accept that chromatography and mass spectrometry are both dynamic rather than static techniques and as a consequence the results that they allow us to derive are based on compromise. In mass spectrometry we are often forced to balance information content, absolute sensitivity and speed. Method design allows us to rebalance the relative importance of each aspect but significant gains in all aspects are only achieved through changes to instrument design[10]. In this respect, I think it is reasonable to expect that improved sensitivity will be the result of innovation in ion management rather than simply an increase in the rate of sampling from the source. The former approach leads to improved detectability while the latter is compromised by the co-sampling of a greater number of matrix related species. While increased sampling of the ion population does not necessarily represent an increase in the signal-to-noise for a target compound it can prove useful in increasing targeted detectability for particularly dilute samples where the matrix is correspondingly simple. We have used the former approach to advantage in the targeted analysis of corticosteroid residues in urine samples (Fig. 6).

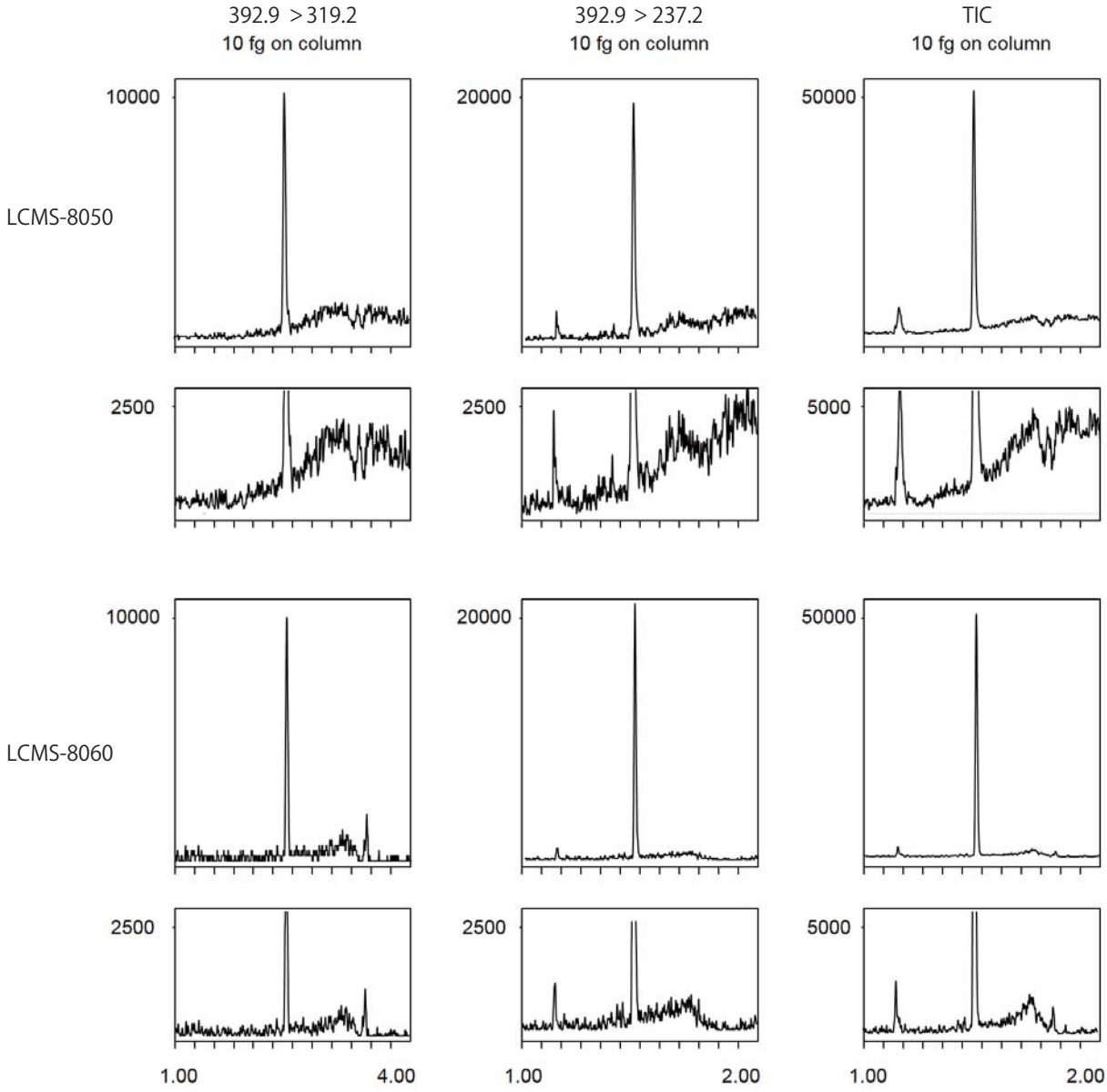


Fig. 6 Ion chromatograms for dexamethasone (10 fg on column) analysed on the Shimadzu LCMS-8050 and LCMS-8060 using identical samples and chromatographic equipment. Data shown are MRM transitions 392.9 > 319.2 and 392.9 > 237.2 with the detector sensitivity adjusted to be equally responsive on both instruments. The expanded portion in each plot shows the real improvement in reducing noise from ion management.

Undoubtedly, HRAM-MS techniques address the signal to noise question in an alternative and powerful way. Instead of relying on the number of ions available to discriminate an analyte from the background, the use of mass filtering provides an effective tool to manage the portion of the population that is detected. We teach that informing power comes at a cost to the method and with HRAM-MS the impact is most commonly linked to compromises in absolute sensitivity and speed. To date, I have not seen a sufficiently objective comparison of TQ and HRAM techniques to know how impactful their differences are on overall method economics but believe there are sufficient publications to support the use of both techniques in a concerted approach to sample analysis.

Where are the innovations to be found that will improve current screening practices? In the case of small molecules, the racing chemists are caught in an endless loop of iterative method change where new compounds are introduced to screening methods.

The class chemistry of drug compounds, ironically one of the major drivers of their separation by HPLC, is no longer an effective means for grouping compounds for testing and so there is a short term reliance on increased multi-analyte targeted methods to meet testing needs. We will continue to look to the instrument designers to provide instruments that are faster scanning, faster switching, more sensitive with extended dynamic range at low concentrations and capable of providing more effective matrix management. Importantly, we will expect those instruments to provide more information about the sample in less time and to assist the racing chemist with data processing. The information may better define the identity of a single analyte or detect and identify a greater number of analytes in a single analysis. With that expectation will come the need for more sophisticated and intelligent data reduction algorithms to better extract information from the acquired data.

Where do large molecules fit into screening programmes?

Even though there is a significant risk that they have been abused, I have deliberately avoided discussing the testing of macromolecules in racing chemistry as this is not my area of expertise. I will take the easy option and only deal with them briefly here. In most racing laboratories, there are now dedicated specialists or groups tasked with developing methods for the detection of peptides and other macromolecules. The very nature of the molecules have meant that LCMSMS techniques are the mainstay of their detection but we also see the tools including immuno-PCR[11] introduced into the testing lexicon. The characteristics that determine a high performing LCMS for small molecule analysis are also relevant to macromolecules. There is a somewhat greater dependence on robustness because sample preparation for peptide and other macromolecular classes usually includes a high level of matrix related components that can be deposited in the instrument source or front-end ion optics. That need can be supported by the ability to do maintenance without venting and open architecture in the pre-assemblies. Again, we have an example of the chemist looking to the instrument designers to produce multipurpose systems that improve productivity.
Macromolecules pose new challenges to drug screening programmes in more than developing methods that allow their detection in urine or blood within a reasonable window of time and determination of their metabolic fate. Forensic toxicology, when applied to racing chemistry, has concentrated its effort on the detection and unequivocal identification of substance residues. The next generation of analysts will address the fundamental challenge of re-educating the regulatory agencies to understand the importance of the macromolecules in their effects, their window of action following administration, the way that they can be administered or abused and the techniques that confirm their presence or use.

Where is forensic toxicology of racing going?

My initial work in the area of forensic toxicology used the principles of Systematic Toxicological Analysis that were also applied to human toxicology. The differences in the case of equine and canine sport were both obvious (for example, different species sometimes give different metabolites) and structural (the industry was interested in participant compliance because effect of substance on performance was not measurable in a meaningful way).
Around the world, different researchers and jurisdictions have attempted to derive threshold concentrations that represent the point at which effect of a substance is minimal or that a level playing field has been reached. The area remains contentious for some and beyond the scope of this discussion because they address the use of the laboratory result rather than their generation.
If I had a crystal ball to predict the future of the industry, it would only be as useful as the link it provided to the innovators currently taking the industry in many different directions. Undoubtedly, the industry will continue to monitor the small molecules that are tested for now because they are effective, stable, inexpensive and easy to use. New substances will continue to be added to the target lists and those who aim to avoid detection or to exploit loopholes in the rules will continue to explore macromolecules, hormone treatments, ergogenic aids and the manipulation of endogenous systems.
Analytically, testing will continue to be supported by instrumentation that provides highly informing data. We understand that to mean mass spectrometry which will continue to be employed as LCMS, GCMS, ICP-MS, LC-ICP-MS and other hybrid techniques that are routinely accessible.
The methodology that is employed needs to continue in its evolution (or return to its historical roots) by looking to specimens other than urine such as blood, saliva and hair for their temporal informing power and ways that information external to the sample of the moment can complement the information that the original specimen provides. Blood may be the specimen of choice for substances that do not survive in detectable quantities in the urine while also providing information more immediate information about the presence of the substance in the body. Hair analysis provides regulators with the opportunity to look outside the usual windows of detection that are associated with blood and urine.
The interest in endogenous compounds and biochemical manipulation also suggests a future direction for small molecule testing in forensic racing chemistry. The question asked of all racing chemists at some point in time is that they comment on the significance of the concentration of a detected substance and whether it (the detected substance) might have had an effect on the animal. Most commentators use this question as a reason to discuss various approaches that might be used to determine threshold concentrations and this topic is consequently regularly discussed between jurisdictions. Thresholds are a management tool for regulators and do not answer the question that the analyst is challenged to answer. If I were to put that another way, in the context of analysis thresholds do not provide greater informing power from the racing chemists work. Rather they provide a framework for managing the racing chemist's results and so should not be seen as a tool to direct the exploration of new opportunities.
What is of interest in the evolution of testing methods is the screening and measuring of drug metabolites and related endogenous substances as compounds of significance. A number of groups have used the perturbation of endogenous metabolites as markers of anabolic steroid[3] and biogenic amine[6] administration and changes in endogenous hydrocortisone can also be reflective of corticosteroid use. These techniques are metabolomic applications and particularly interesting in that they have the power to show disruption or change to an animal's biochemistry. They are therefore able to demonstrate an effect on the racing animal's physiology and such demonstrations can be powerful statements that reinforce the appropriateness of a known substance test when the result is included in a brief of evidence.
Of far greater significance to the development of new testing approaches, the use of characterised metabolomic models offers an opportunity to use profiling of endogenous compounds as a screening tool to indicate the possible use of one or more of a class of prohibited substance. For example, a well characterised model for the arachidonic acid cascade (prostaglandin and leukotriene metabolites) might provide a facile means to indicate the use of one or more of the many different classes of anti-inflammatory drugs that are now available.
In such a model, laboratory managers would look to the very small percentage of positive samples recorded in current sample populations as the protection against a significant confirmatory testing commitment for the laboratory flowing from confirmatory testing that would need to respond to an indicative metabolomics screen.
Whichever way the industry proceeds, it is clear that the continued efforts of the innovators in the industry will continue to be of importance and that their ability to drive growth with be dependent on instrument manufacturers like Shimadzu to break new ground in instrument capabilities and techniques.

References

[1] Wynne, P.M., Batty, D.C., Vine, J.H. and Simpson, N.K.J. (2004). A review of approaches to SPE of equine urine. *Chromatographia*, 59 (4/5), S50-S61.

[2] Wynne, P.M., Vine, J.H. and Amiet, R.G. (1998). The metabolism and urinary excretion of 2-amino-4-methylpyridine in the horse. *Proc 12th Int Conf rac Anal Vet.* 166-170.

[3] Wynne, P.M., Vine, J.H. and Amiet, R.G. (2004). 3-Methoxytyramine as an indicator of dopaminergic manipulation in the equine athlete. J Chromatogr. B., 811, 93-101

[4] McKinney, A.R., Richards, S.L., Cawley, A.T., Keledjian, J. Wynne, P.M. and Suann, C.J. (2016). Catechol-O-methyltransferase inhibitors and the equine 3-mthoxytyramine threshold. Proc 20th *Int Conf rac Anal Vet.* 295-299.

[5] Glowacki, L., Vine, J and Wynne, P. (2012). Structure-retention relationships for basic drugs separated on different GC phases. *Proc 18th Int Conf rac Anal Vet.* 470-477.

[6] Viljanto, M., Scarth, J., Hincks, P., Hillyer, L., Cawley, A., Suann, C., Noble, G., Walker, C.J., Kicmane, A.C. and Parkine, M.C. (2017). Application of testosterone to epitestosterone ratio to horse urine – a complementary approach to detect the administrations of testosterone and its pro-drugs in Thoroughbred geldings. *Drug Test Analysis,* 1328-1336.

[7] Ross, C., Grieves, N. and Wynne, P. (2016). Systematic screening for drugs in urine using ultrafast-GCMSMS with simultaneous non-targeted EI-scan and targeted MRM modes. *Proc 20th Int Conf rac Anal Vet.* 557-563.

[8] Levi, M., Moreau, S. and Wynne, P. (2016). Approaches to the analysis of steroid residues in urine and plasma using HESI and LCMSMS. *Proc 20th Int Conf rac Anal Vet.* 376-382.

[9] Wynne, P., Van, N. and Grieves, N. (2016). Extending the scope of triple quadrupole LCMS for structure elucidation. *Proc 20th Int Conf rac Anal Vet.* 564-569.

[10] Wynne, P.M. (2018). Corticosteroids and other polar drugs as probes of triple quadrupole mass spectrometer sensitivity. *Proc 21st Int Conf rac Anal Vet.* 354-361.

[11] Timms, M., Hall, N. and Steel, R. (2018). Detection of CJC-1295 by immune-PCR: A platform technology for the detection of doping proteins in equine racing. *Proc 21st Int Conf rac Anal Vet.* 166-174.

About the author

Dr. Paul Wynne is currently the manager of Monash University's Medicines Manufacturing Innovation Centre in Parkville, Victoria, Australia. He is a graduate of the Royal Melbourne Institute of Technology (now RMIT University) with a Master's degree in Organic Photochemistry and a PhD in Forensic Chemistry and Toxicology. Paul spent 15 years as the Senior Research Chemist and Principal Scientist at Racing Analytical Services Limited, the Official Analyst to racing jurisdictions in Victoria, Tasmania and South Australia, 6 years as Chromatography Manager with SGE Analytical Science and then 5 years as a Technical Specialist and Regional Manager with Shimadzu Scientific Instruments (Oceania) Pty. Ltd., Australia. He has lectured in the pragmatic use of instrumental analysis and method design for the past fifteen years.
Paul has published over 80 peer reviewed papers concentrating on Forensic Racing Chemistry, borderline substances and nutraceuticals.

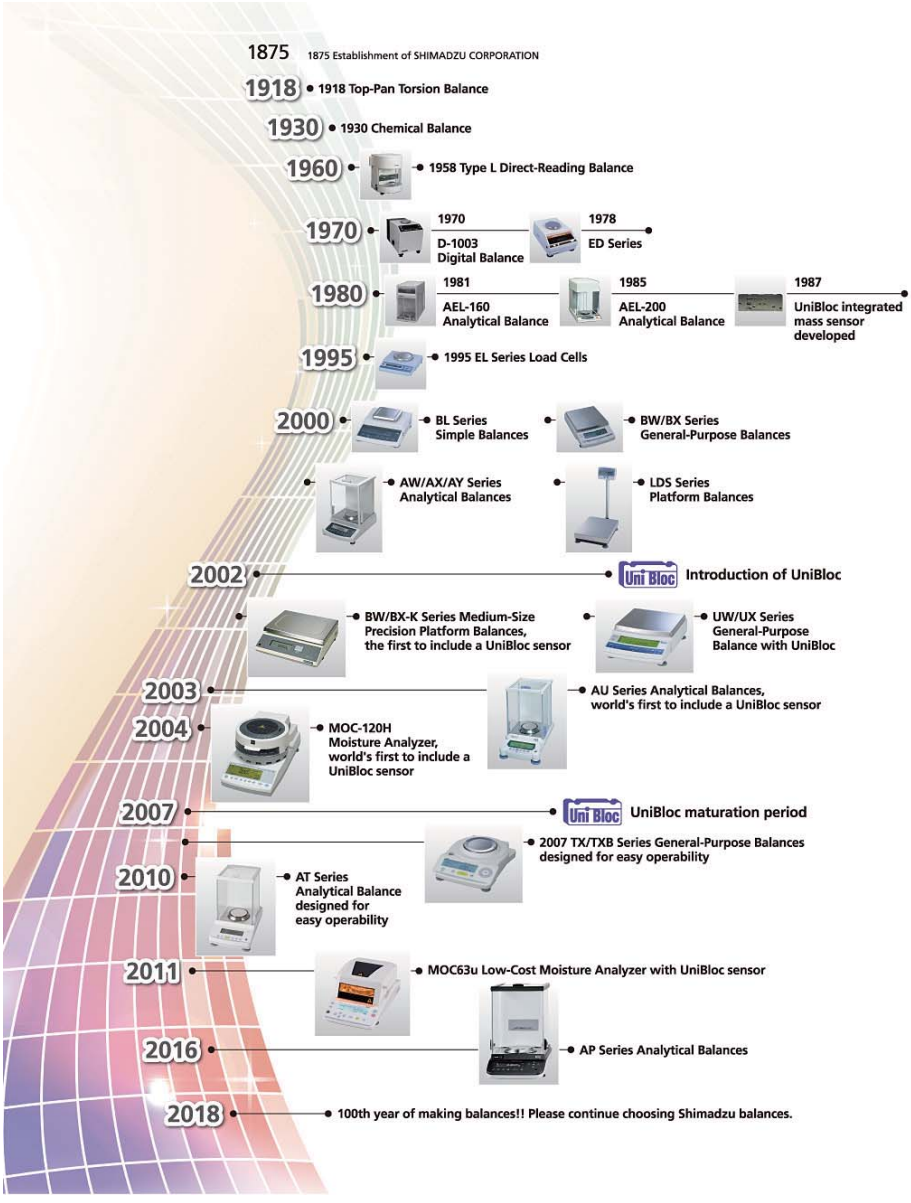
100th Anniversary of Balances

Providing Balances and Scales for 100 Years

Shimadzu Corporation is proud to announce its 100th anniversary of scale manufacturing, which follows the 100th anniversary of manufacturing testing machines, celebrated last year.

We have been contributing to society with technologies since our foundation in 1875. In 1918, we started our balances and scales business with the launch of an experimental scale. Throughout our history, we have provided solutions at the highest quality level, and continue to utilize our extensive experience and trustworthy measurement technology, including our core UniBloc technology, to manufacture high-performance products for diverse fields. Our customer-first dedication to high technologies is not only the hallmark of what we have been doing but also the principle that guides us into the future. With this as our guide, we will continue to innovate for the next 100 years and beyond.
We would like to express our gratitude for your continuous patronage.

100th BALANCE ANNIVERSARY



The AIM-9000 Infrared Microscope and IRSpirit Fourier Transform Infrared Spectrophotometer Win Red Dot Design Awards for Product Design 2018

Two Shimadzu analytical instruments, the AIM-9000 infrared microscope and the IRSpirit Fourier transform infrared spectrophotometer, have been awarded the Red Dot Design Award for Product Design 2018, a renowned design prize in Germany.
Although Shimadzu Industrial Systems Co., Ltd. won this prize for the VESTA vacuum and pressurized sintering furnace in 2013, this is the first time Shimadzu Corporation products have received the Red Dot Design award.
The Red Dot Design award is a worldwide design award supported by Design Zentrum Nordrhein Wastfalen in Germany. Excellent designs are selected from amongst ones advertised for the past two years. The high-tech Shimadzu products were among more than 6,300 submissions from 59 countries. The products will be on display in the Red Dot Design Museum in Essen for one year.

Brief descriptions of the two products are as follows:

AIM-9000 Infrared Microscope

The AIM-9000 infrared microscope is equipped with a high-speed automatic measurement system for microscopic contaminants, enabling failure analysis, such as confirming foreign substances in pharmaceutical tablets or contaminated electronic substrates that cause contact failure. The best thing about the AIM-9000 is the ability to automatically perform procedures for determining the measuring points, fixing the measurement area, and analyzing undefined samples with a successful achievement of a 30,000:1 S/N ratio.

IRSpirit Fourier Transform Infrared Spectrophotometer Series

IRSpirit series features infrared lights that anticipate the chemical structures based on analyses of substances. In addition, the world's smallest and lightest instrument, with a size of 390mm in width and 250mm in depth and weight of 8.5 kg, makes it a highly portable system with exceptional versatility. An analysis assistant program enables easy operation of 23 types of confirmation tests and contaminant analyses.

The Red Dot Design Award Ceremony took place in the Aalto-Theater Essen on 9th July, 2018. Approx.1,200 international guests participated in the ceremony and celebrated Winners' design achievements. The primary designers, Ms. Kyo and Ms. Ihara, of the General Design Centre, Design Unit, attended to receive the prestigious award.
Pictures of the main venue of the event, display items, and award-winning designers can be seen below.
For more information, please click here: https://www.red-dot.org/about-red-dot/news/red-dot-gala-2018/

Red Dot Design Museum, the biggest museum of modern design in the world.

Award Winners: Ms. Kyo (Left) and Ms. Ihara (Right)

AIM-9000 Infrared Microscope (Right)

IRSpirit Fourier Transform Infrared Spectrophotometer Series

SIC's cutting-edge joint research with international partners

1. Introduction

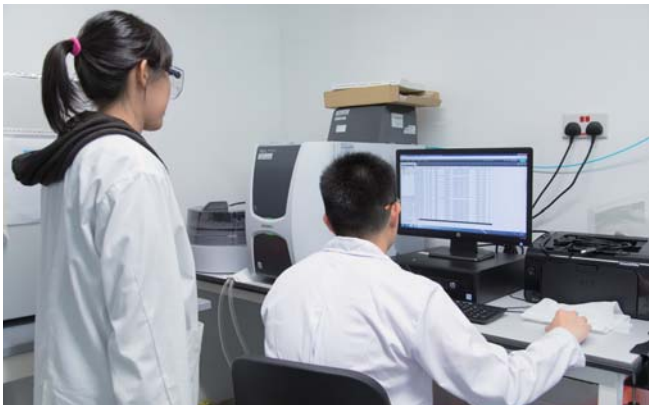
The Shimadzu Innovation Centre (SIC) has found a valid and feasible way to both develop its own commercial products and engage in collaborative research. Recently, SIC worked on water contamination caused by an outburst of toxic algal bloom in collaboration with members of the National University of Singapore (NUS). Since Shimadzu's foundation in 1875, it has provided a wide range of technologies for applications in various fields, developments that have made a big impact worldwide. However, Shimadzu has found it challenging to provide a solution for the supply of clean water.

2. Background of water analysis

The difficulty that SIC and NUS faced are algal blooms, global public health risks caused by phosphate. Phosphate is generated mainly by agricultural runoff and sewage and triggers a surge in phosphate consolidation. Consequently, it causes extremely harmful compounds that pollute water and prevents people from having access to clean drinking water.
Conventional phosphate detection systems can predict the possible locations where algal brooms are likely to happen, but low sensitivity prevents responders from having ample time for countermeasures before algae proliferation.
To address this, SIC and NUS proposed a solution that combines the use of diffusive gradients in thin films (DGT), a technique for contaminant detection in water and soil, with Shimadzu's integral technologies for water quality analyses. This combination allows for on-site analysis and quick, high-sensitivity detection of phosphate levels.

3. Shimadzu Innovative Centers

This collaborative research between SIC and NUS is the outcome of a new Shimadzu partnership strategy. Its most recently established SIC, the Asia Pacific Innovation Centre in Singapore, opened in 2017. SICs bring Shimadzu engineers and collaborators like NUS together in order to create prototypes and commercial products. The three other SICs, located in the United States, China and Europe, function in the same way but may take different approaches related to, for example, applications, innovation, and political priorities.



4. A new approach through collaborative efforts

The collaborative research with NUS has inspired both parties to a new idea of a compact, wireless, low-priced device enabling transmission of real-time data to a user's smartphone utilizing solar energy. At the same time, Shimadzu will attempt to modify sensors in order to detect other water contaminants with the goal of an all-in-one instrument. This moment firmly establishes Shimadzu's new approach to product development and to building relationships with its partners. These interactive R&D initiatives will continue to expand through the SICs, leading to new technologies in a faster, more efficient manner.

New Products

Nexera Mikros

(Microflow Liquid Chromatography Mass Spectrometry System)

Micro Flowrate Compatible Liquid Chromatograph Mass Spectrometer, Provides the Pharmaceutical Industry with High Sensitivity, Durability, and Ease of Use

Micro:
Above and Beyond Nano

Click here>

The Nexera Mikros improves operability and durability in LC-MS analyses, enabling better operational efficiency and shorter research and development time.

Features

- Improvement in durability and operability of LC-MS systems
- High-sensitivity analyses in shorter times
- Low detection limits with microflow

Smart Forensic Database Ver. 2

(Forensic Database for GC-MS/MS Analysis)

Supporting the creation of MRM methods for forensic toxicological substances

Click here>>

This database supports the creation of high-sensitivity GC-MS/MS MRM methods for forensic toxicological substances. The automated generation of MRM methods makes measurement settings in the MRM program easy, eliminating the need for time-consuming configurations.

Features

- Supports simultaneous analyses of forensic toxicological substances
- Detects forensic toxicological substances with improved sensitivity
- Automatic creation of MRM methods

UV-1900

(UV-VIS Spectrophotometer)

Offering the Industry's Fastest Level Scan Function and LabSolutions UV-Vis Control Software

Navigate Your Way

Click here>>

The UV-1900 enables high-speed data acquisition with an ultra-scan function. Moreover, it incorporates diverse regulatory compliance functions and makes integrated data management with all Shimadzu instruments possible.

Features

- Easy operation via on-screen user interface
- High-accuracy, high-speed quantitative analyses
- Compatible with various regulations and guidelines
- A wide range of measurement modes

LCMS-9030

(Q-TOF Liquid Chromatograph Mass Spectrometer)

Enhancing Shimadzu's mass spectrometry platform

Effortless Performance

Click here>>

Shimadzu's first quadrupole time-of-flight (Q-TOF) mass spectrometer combines the high-speed performance and ion assemblage capacities from the triple quadrupole LCMS-8000 series with powerful, up-to-date TOF technology. This product provides exceptional mass accuracy, sensitivity and resolution, and a precision-engineered temperature control system ensures long-term stability of mass accuracy.

Features

- High-speed performance and ion convergence capabilities
- Excellent mass accuracy with high sensitivity and high resolution
- High-accuracy temperature control system

Find us on

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/

Printed in Japan, September, 2018