

# Case Study

## Analysis of Equine Doping Using TurboFlow Technology and Multiplexing with LC-MS/MS

Singapore Turf Club Case Study

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Dr. Shawn Stanley,  
Chief Analyst, Singapore Turf Club



### Introduction

The Singapore Turf Club ran its first race in 1842, for the then-sizable purse of \$150. Today hundreds of millions of dollars are won and lost at the track every year. With that kind of money at stake, the fair outcome of a race must be beyond question. As chief analyst at the Singapore Turf Club's testing lab, Dr. Shawn Stanley is tasked with making sure there is never a doubt. Dr. Stanley uses some of the most advanced mass spectrometry technology in the world to test more than 15,000 horse blood and urine samples each year.



“There’s a lot of money in horse racing, and the legal environment is such that we have to ensure whatever we make from the lab are defensible,”

Dr. Stanley said. “Our methods, our technology and our results all have to be solid. We don’t want a situation where we pull a horse out of a race based on a test result and then later on it turns out the result can’t be confirmed.”

Dr. Stanley’s team works under intense pressure on race days. They have just two hours before each race – during which they must test 12 to 13 samples, confirm any sample finding that is suspicious, and, if necessary, notify officials to pull a horse from a race. For Dr. Stanley’s lab to succeed, he requires not just the highest accuracy, but also speed and ease-of-use from his testing system.

### The Challenge

Screening for performance-enhancing drugs in horses is an incredibly challenging problem because race officials are usually looking for illegal steroids that have similar molecular structure as naturally occurring steroids in animals. Further complicating the testing process is the fact that horse urine and blood are both complex, dirty matrices – making the separation especially challenging.

Dr. Stanley previously used a mass spectrometry system that required liquid-liquid extraction, but said he needed a system that was both faster and more reliable. Dr. Stanley adopted the Thermo Scientific Transcend TLX-4 system – the only truly independent, parallel, multichannel U-HPLC system. The Transcend™ system, powered by Thermo Scientific TurboFlow technology, provides advanced capability compared to traditional LC separation front-end systems, offering high throughput, online sample extraction, superior data quality and ease-of-use.

### Sample Preparation

Sample preparation was the biggest bottleneck in Dr. Stanley’s lab and a major reason he became interested in the TLX-4 system.

Transcend systems save time because they allow the user to inject an untreated sample, like plasma or urine, directly into the system, eliminating time-consuming sample prep processes such as liquid-liquid extraction, solid-phase extraction and protein precipitation. No preparation is necessary because Transcend uses an innovative TurboFlow™ method to separate analytes from biological fluids prior to MS/MS analysis.

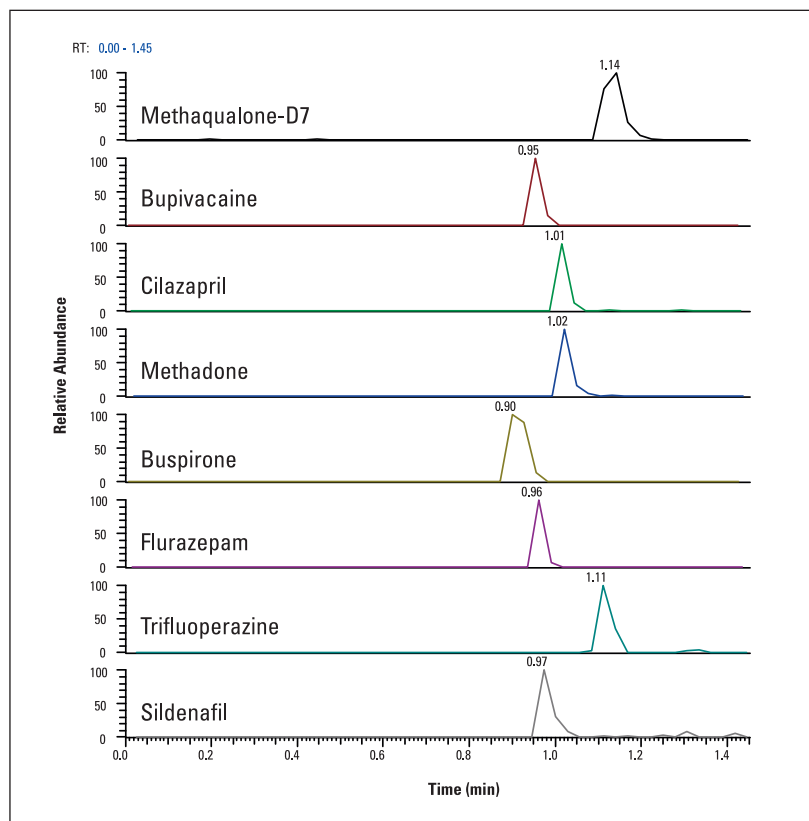
“What we liked about the Transcend system is that it offered an opportunity to reduce our sample preparation down to a limited few steps,” Dr. Stanley said. “We didn’t have to wait for a sample to dry down; we could even put the sample on directly, although for robustness we do a little sample preparation in the pre-race analysis.”



Dr. Stanley estimates the Transcend system shaves 20 minutes off the two hours it used to take him to run a batch of samples – a huge savings on a typical race day consisting of a dozen races or more. The decision to pull a horse or keep it in the race can have huge legal implications for the track. Dr. Stanley said he needs the best data he can get to support his calls.

“Now we have the luxury of spending more time confirming a sample that is flagged. We don’t have to make decisions instantly, so there’s a lot less pressure,” Dr. Stanley said. “Before, we were running so short of time that if there were any hiccups in the second stage of confirmation, it might be too late to call a horse out of the race.”

**The Transcend TLX-4 system with the Thermo Scientific TSQ Quantum Ultra mass spectrometer provided a 30 second data window for eight antibiotic calibration standards.**



## Multiplexing

Because of the volume of screening work in Dr. Stanley’s lab, multiplexing is a necessity. The multiplexing capability of the Transcend system was a key reason he adopted it.

“Our previous instrument ran four samples at once, so it was always sampling each channel 25 percent of the time, which decreases your sensitivity a lot; you lose more than 50 percent compared to just running a single channel,” Dr. Stanley said.

The Transcend system delivers a huge increase in throughput, enabling users to run two or four different methods simultaneously on one mass spectrometer.

These unique capabilities do not come at the cost of data quality. Because the operation of each multiplexed LC system is staggered and parallel, the mass spectrometer is dedicated solely to a single sample stream during the critical elution step, maintaining data quality and sensitivity throughout the process. The TLX-4 system quadruples the throughput of a single channel system, reducing typical mass spectrometer idle time from 75 percent to 4 percent.

## Online Operations

With a staff of 19 and several different groups of technicians working on the system, Dr. Stanley identified ease-of-use and the ability to unify all online operations on a single software platform as critical factors in his decision.

All of the Transcend system online operations are controlled by Thermo Scientific Aria software – including multiplexing, pump, valve and autosampler operation.

“From the first day we had the system up and running, we were getting results. Literally, we were using it in eight hours and understood it pretty well,” Dr. Stanley said. “The Aria software made it simple to play around with the various parameters, and the graphic interfaces were easy to follow.”

## Conclusion

The Transcend system reduced sample preparation time and increased mass spectrometry throughput for the Singapore Turf Club. In addition, the system virtually eliminated ion suppression by removing 99 percent of all endogenous phospholipids.

The opportunity to purchase a complete solution that is ready to run out of the box is one reason Singapore Turf Club turned to Thermo Scientific technology. “Other companies said, ‘Buy a bit of this, and a bit of this and this, and we’ll put it together for you and make it work,’” Dr. Stanley said. “But we can’t afford to spend the next two years doing a research project. These are frontline instruments and we needed something with the kind of reliability Thermo Fisher Scientific offers.”

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