Analysis of doping agents using ultrafast LC-MS/MS with scheduled MRM

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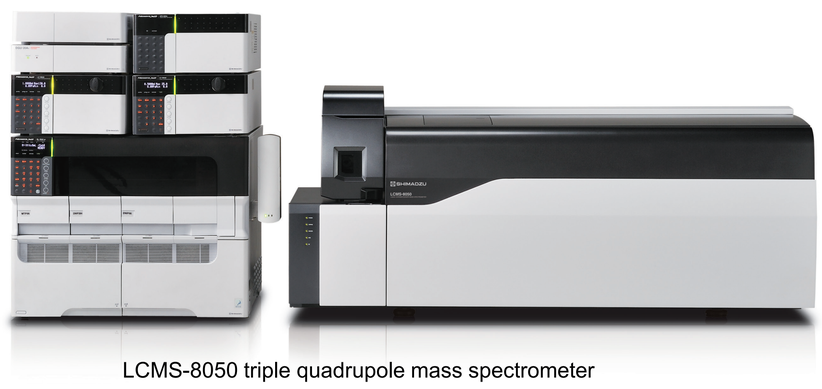
1. Introduction

Faster, higher, further - doping accompanies sports already for many centuries. But as it was not possible to detect the illegal substances at that time, the first doping case was discovered in 1812 - only because the culprit was caught in the act.

In general, doping refers to the use of banned performance-enhancing drugs, or the use of banned methods to improve performance. But doping not always means improvement of performance. In horse racing, for example, terms such as negative doping, which is doping to defeat, are an issue.

In the past the attitude “Allowed is, what is not found” predominated. Nowadays improved analytical methods allow the detection of even the slightest traces of doping agents in blood and urine. Thus, the analytical possibilities of the different labs are crucial for the detection of a substance. Here we show the advantage of an ultrafast MS technique with excellent sensitivity when analyzing horse doping agents.



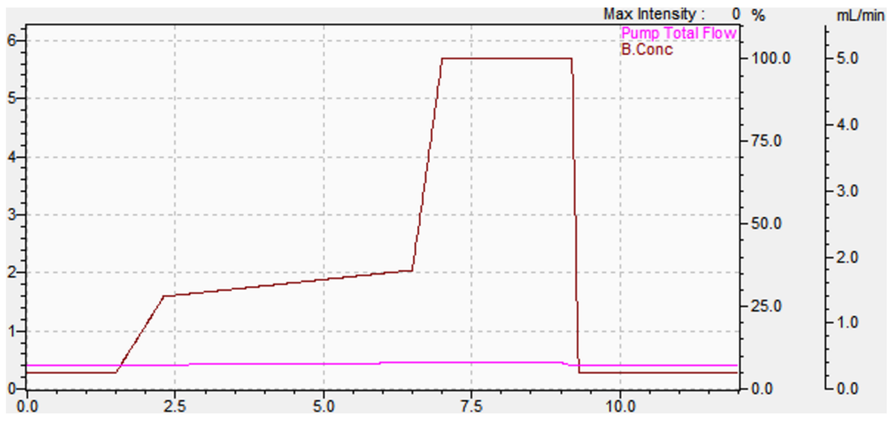
LCMS-8050 triple quadrupole mass spectrometer

2. Materials and Methods

Real urine samples from a horse doping laboratory were tested after sample pretreatment for various corticosteroids and other small molecules. The samples were analyzed using the high sensitivity triple quadrupole mass spectrometer LCMS-8050 coupled to a NEXERA X2 UHPLC (Shimadzu, Japan) operating in scheduled MRM mode with fast polarity switching (5 msec) for the detection of positively and negatively charged ions in one run. To corroborate the data quality of the ultrafast scheduled MRM analysis two different screening methods containing MRM transitions for either 13 components (resulting in 26 MRMs) or 127 components (resulting in 254 MRMs) were compared. In addition the repeatability of MRM only experiments compared to MRM experiments including a synchronized survey scan (data dependent product ion scan) were investigated.

2-1. Analytical Conditions

UHPLC		
LC system:	Nexera X2 (Shimadzu, Japan)	
Analysis Column:	Acquity C_{18} (2.1 mm I.D. x 100 mm L., 1.7 µm)	
Mobile Phase A:	Water + 0.1 % Formic acid	
Mobile Phase B:	Acetonitrile + 0.1 % Formic acid	
Column Temperature:	25 °C	
Injection Volume:	10 µL	
MS		
MS system:	LCMS-8050 (Shimadzu, Japan)	
Ionization:	HESI (positive/negative)	
Nebulizing Gas Flow:	3.00 L/min (N_2)	
Drying Gas Flow:	5.00 L/min (N_2)	
Heating Gas Flow:	15.0 L/min (Air)	
DL Temperature :	250 °C	
Block Temperature :	450 °C	
Interface Temperature :	300 °C	
LC Gradient		
Time (min)	Flow rate (mL/min)	%B
0.00	0.35	5
1.50	0.35	5
2.30	0.35	28
6.50	0.35	36
7.00	0.35	100
7.20	0.40	100
9.00	0.40	100
9.20	0.35	100
9.30	0.35	5
12.00	0.35	5



3. Results

3-1. Data comparison

The chromatograms to evaluate the data quality when using ultrafast scheduled MRM methods were obtained from a urine extract containing 1 pg/mL of 13 different components. Comparison between acquisition with 13 events (26 MRM) and 127 events (254 MRM) show that even at high speed with 1ms dwell time and ultra fast polarity switching the sensitivity is at the same level (Fig. 1, Fig. 2).

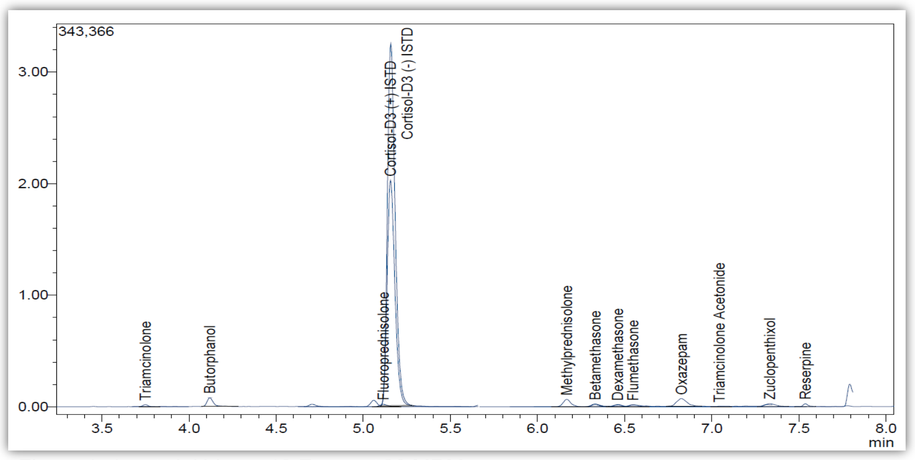


Figure 1. 1 pg/mL Urine – 13 Events / 26 MRMs

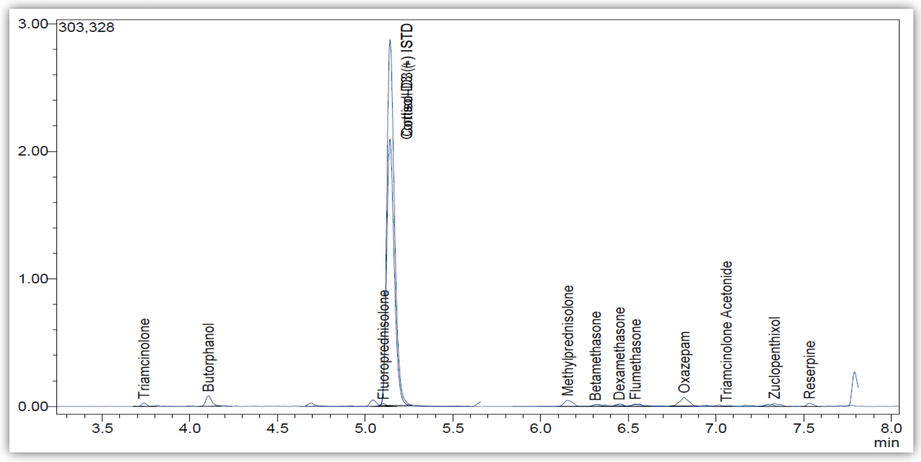


Figure 2. 1 pg/mL Urine – 127 Events / 254 MRMs

The detailed assessment of a less intense peak (e.g. Triamcinolone) proves the consistent high quality of data despite a strongly raised number of MRMs in the method (Fig. 3)

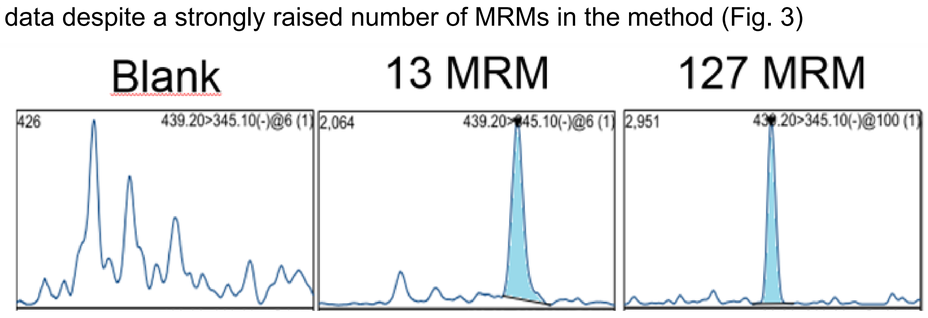


Figure 3. Data comparison 13 MRMs vs 127 MRMs

3-2. Repeatability

A standard solution equivalent to an extracted sample at 2 pg/mL was injected 6-fold. An acquisition method using MRM mode only was compared with an acquisition mode combining MRM mode with a synchronized survey product ion scan at 30000 Da/sec.

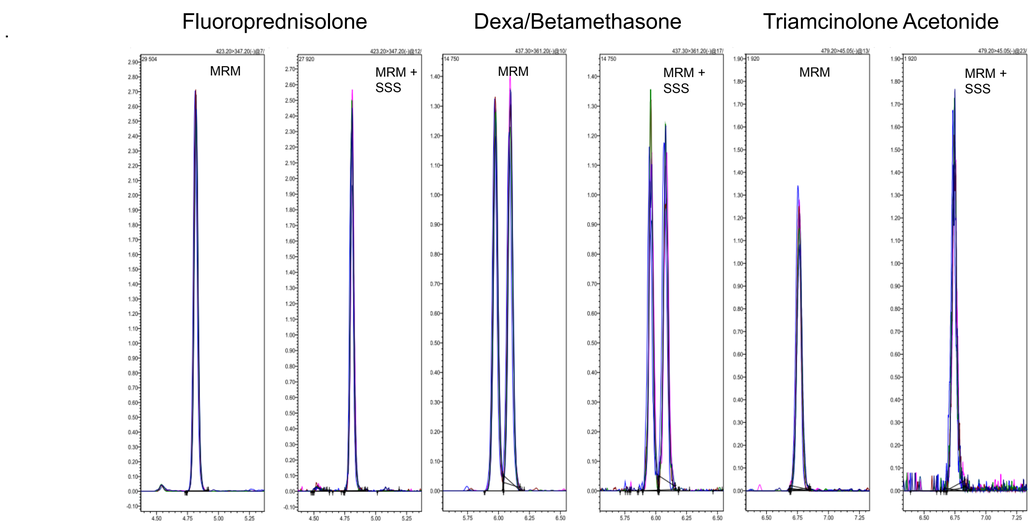


Figure 4. Examples for repeatability MRM only and MRM + synchronized survey scan.

Compound	Peak area %RSD MRM only	Peak area %RSD MRM + Survey scan
Cortisol (neg)	2.2 %	2.7 %
Cortisol (pos)	5.1 %	7.5 %
Butorphanol	0.8 %	0.6 %
Reserpine	3.1 %	3.8 %
Oxazepam	2.0 %	3.3 %
Zuclopenthixol	2.0 %	2.3 %
Bethamethasone	2.5 %	4.3 %
Dexamethasone	2.1 %	3.0 %
Flumethasone	3.5 %	4.5 %
Methylprednisolone	1.7 %	2.9 %
Fluoroprednisolone	2.4 %	4.5 %
Triamcinolone Acetonide	4.1 %	3.6 %
Triamcinolone	9.3 %	4.3 %

4. Conclusion

Independent from the number of MRMs or simultaneously performed synchronized survey scans the LCMS-8050 coupled to a Nexera X2 system provides excellent sensitivity with high data quality in scheduled MRM mode with ultra fast polarity switching (5 msec) for the detection of positively and negatively charged analytes in one run.