Validation and uncertainty evaluation of the identification of doping agents in sport by GC-MS/MS

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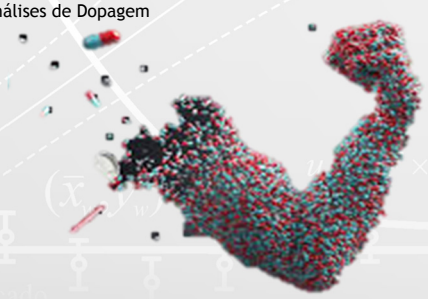

Overview

1. Problem
2. RRT and AR distribution
3. Setting criteria for RRT and AR values
4. WADA criteria for analyte identification
5. Comparison of identification criteria
6. Conclusion

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1. Problem

• The identification of doping agents in urine samples by GC-MS-MS is supported on the agreement between Relative Retention Times, RRT, and Ion Abundance Ratios, AR, of the analyte from a calibrator and a sample peak;

• The criteria for the agreement between RRT and AR is set in WADA documents independently of observed performance of the GC-MS-MS.
(WADA guidelines are mandatory)

WADA - World Anti-Doping Agency.

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2. RRT and AR distribution

• Although retention times and ion abundances collected in different GC-MS injections have a normal distribution, the ratio between RT of peaks of the same chromatogram or the ratio of abundances of ions of the same mass spectrum, can be not normally distributed.

Replica	*e* (a.u.)	*a* (a.u.)	*g* (a.u.)	AR_{ea}	AR_{ga}
1	121630	147887	96261	0.822	0.651
2	110510	141073	95079	0.783	0.674
3	104483	136593	87475	0.765	0.640
4	87209	129136	74081	0.675	0.574
5	91776	129224	77586	0.710	0.600
(...)					
Dist. (distribution)	Normal	Normal	Normal	Not-Normal §	Not-Normal §

§ - Exact distribution depends of abundances mean values, standard deviations and correlations.

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[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	0.783	0.674
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§ - Exact distribution depends of abundances mean values, standard deviations and correlations.

• The difference between a pair of RRT or AR of two consecutive injections tend to be normally distributed, but it is safer not to use normal statistics.

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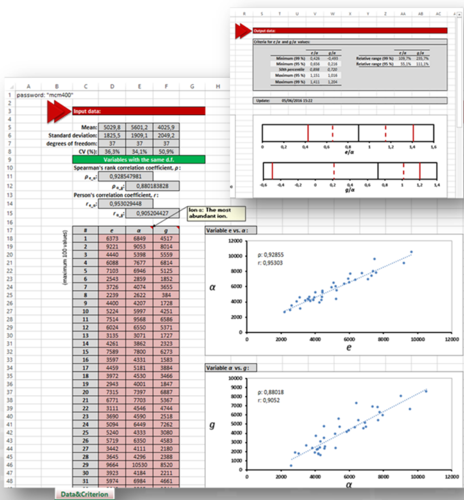
3. Setting criteria for RRT and AR values

• A tool was developed, based on Monte Carlo Simulations, to estimate intervals of RRT, AR and differences of pairs of RRT and AR.

The intervals were estimated from the mean and standard deviation of RT or Ion Abundances, and from the correlation of pairs of these values.

» The confidence interval of RRT and AR difference, sets the True Positive Results Rate (TP).



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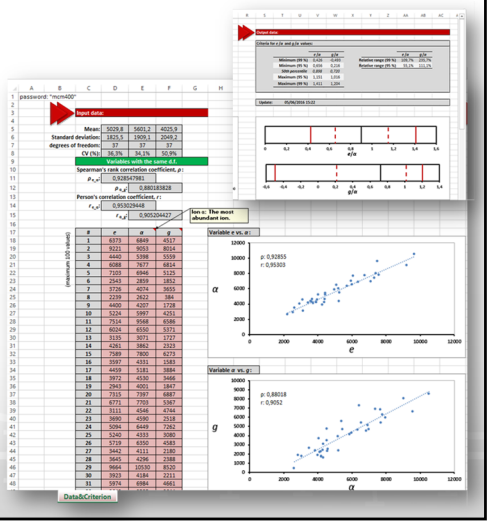
3. Setting criteria for RRT and AR values

• A tool was developed, based on Monte Carlo Simulations, to estimate intervals of RRT, AR and differences of pairs of RRT and AR.

The False Positive results rate (FP) from AR is estimated from models of the signal noise.

» The FP is the probability of noise producing an AR difference within the acceptance limits of the AR difference.



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3. Setting criteria for RRT and AR values

• This tool was used in the identification of doping agents in urine by GC-MS-MS.

The uncertainty of identification performed using this tool (i.e. TP, FP and LR) was compared with the uncertainty of identifications performed using WADA criteria and a less strict criteria used at screening stage.

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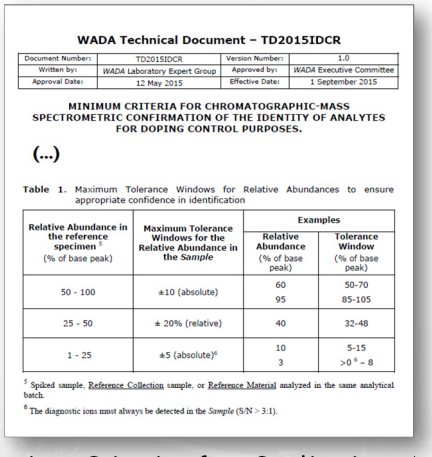
4. WADA criteria for analyte identification

WADA defines criteria for compounds identification [1]:



WADA Technical Document – TD2015IDCR

Document Number:	TD2015IDCR	Version Number:	1.0
Written by:	*WADA* Laboratory Expert Group	Approved by:	*WADA* Executive Committee
Approval Date:	12 May 2015	Effective Date:	1 September 2015

MINIMUM CRITERIA FOR CHROMATOGRAPHIC-MASS SPECTROMETRIC CONFIRMATION OF THE IDENTITY OF ANALYTES FOR DOPING CONTROL PURPOSES.

(…)

Table 1. Maximum Tolerance Windows for Relative Abundances to ensure appropriate confidence in identification

Relative Abundance in the reference specimen [5] (% of base peak)	Maximum Tolerance Windows for the Relative Abundance in the *Sample*	Examples	
		Relative Abundance (% of base peak)	Tolerance Window (% of base peak)
50 - 100	±10 (absolute)	60 95	50-70 85-105
25 - 50	± 20% (relative)	40	32-48
1 - 25	±5 (absolute)[6]	10 3	5-15 >0 [6] – 8

[5] Spiked sample, Reference Collection sample, or Reference Material analyzed in the same analytical batch.

[6] The diagnostic ions must always be detected in the *Sample* (S/N > 3:1).

1. WADA,Identification Criteria for Qualitative Assays,TD2015IDCR, 2015.

5. Comparison of identification criteria

Table 1: Analyte identification criteria at the Minimum Required Performance Level (MRPL).

Analyte	RRT difference (min)		AR difference window (relevant ions)		
	CoVaras (c.l.:95 %)	WADA and Screening	CoVaras (c.l.: 95%)	WADA	Screening
Triamterene	±0.0052	±0.012	-0.066 to 0.067	±0.052	±0.26
Modafinil	±0.0073	±0.0063	-0.427 to 0.433	±0.075	±0.38
Amiloride	±0.0080	±0.0049	-0.207 to 0.206	±0.088	±0.22
Epimentendiol	±0.0061	±0.0074	-0.038 to 0.039	±0.0048	±0.048
5βTHMT	±0.0052	±0.0095	-0.382 to 0.391	±0.085	±0.21
6β-idroximetandienone	±0.0073	±0.012	-0.091 to 0.092	±0.0054	±0.054

RRT – Relative Retention Time;
AR – Ions Abundance Ratio of relevant fragments of the mass spectrum.

5. Comparison of identification criteria

Table 2: Likelihood ratio from different identification criterion at MRPL.

Analyte	RRT (LR=TP(%)/FP(%))		AR (LR=TP(%)/FP(%))		
	CoVaras (c.l.:95 %)	WADA & Screening	CoVaras (c.l.: 95%)	WADA	Screening
Triamterene	95 = = 95/1	99.99 = = 99.99/1	9.5×10^4 = = 95/(1×10^{-3})	9.79×10^4 = =97.9/(1×10^{-3})	9.95×10^4= =99/(1×10^{-3})
Modafinil	95 = = 95/1	99.6 = = 99.6/1	25.4 = = 95/3.7	4.87×10^4= =48.7/(1×10^{-3})	1.34×10^4 = =94/(7×10^{-3})
Amiloride	95 = = 95/1	98.66 = = 98.66/1	9.5×10^4 = = 95/(1×10^{-3})	8.22×10^4 = =82.2/(1×10^{-3})	9.54×10^4= =95/(1×10^{-3})
Epimetendiol	95 = = 95/1	99.8 = = 99.8/1	9.5×10^4 = = 95/(1×10^{-3})	9.79×10^4 = =97.9/(1×10^{-3})	9.75×10^4= =98/(1×10^{-3})
5βTHMT	95 = = 95/1	99.97 = = 99.97/1	3.26 = = 95/29	5.35×10^4 = =53.4/(1×10^{-3})	4.31×10^4= =86/(2×10^{-3})
6β-hidroximetandienone	95 = = 95/1	99.6 = = 99.6/1	26.8 = = 95/3.5	8.19×10^4 = =81.9/(1×10^{-3})	1.7 = = 85/50

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5. Comparison of identification criteria

Table 3: Likelihood ratio and False Negative Results Rate (FN) of identifications based on RRT and AR at the MRPL.

Analyte	LR=LR(RRT)×LR(AR)			FN (%) from AR		
	CoVaras	WADA	Screen	CoVaras	WADA	Screen
Triamterene	9.0×10^6	9.8×10^6	1.0×10^7	5 %	2.1 %	0.51 %
Modafinil	2.4×10^3	4.9×10^6	1.3×10^6	5 %	51 %	6.5 %
Amiloride	9.0×10^6	8.1×10^6	9.4×10^6	5 %	17 %	4,6 %
Epimetendiol	9.0×10^6	9.8×10^6	9.7×10^6	5 %	2.1 %	2.5 %
5βTHMT	3.0×10^2	5.3×10^6	4.3×10^6	5 %	46 %	14 %
6β-hidroximetandienone	2.6×10^3	8.1×10^6	1.7×10^2	5 %	18 %	15 %

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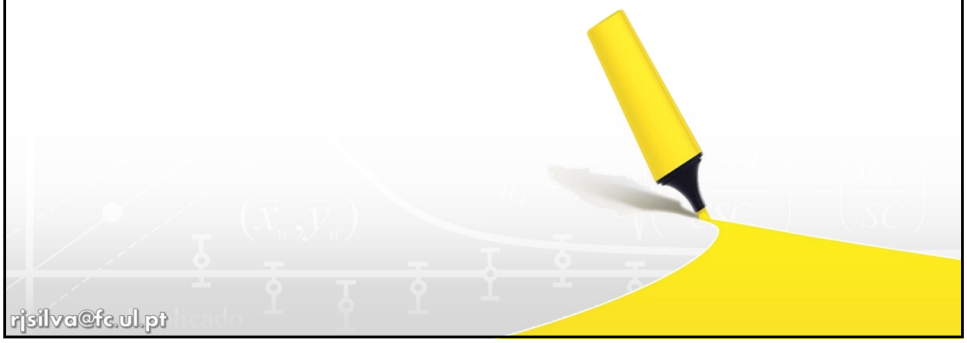
6. Conclusions

• The developed statistical models for RRT and AR allow to conclude that WADA criteria are safe for positive results but can be associated with large FN;

• There are tools available to reduce the FN but it will increase FP. The increase of FP must be under control.



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