Sport Doping Screening in Biological Matrices by Multi-Dimensional LC-QToF

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Abstract

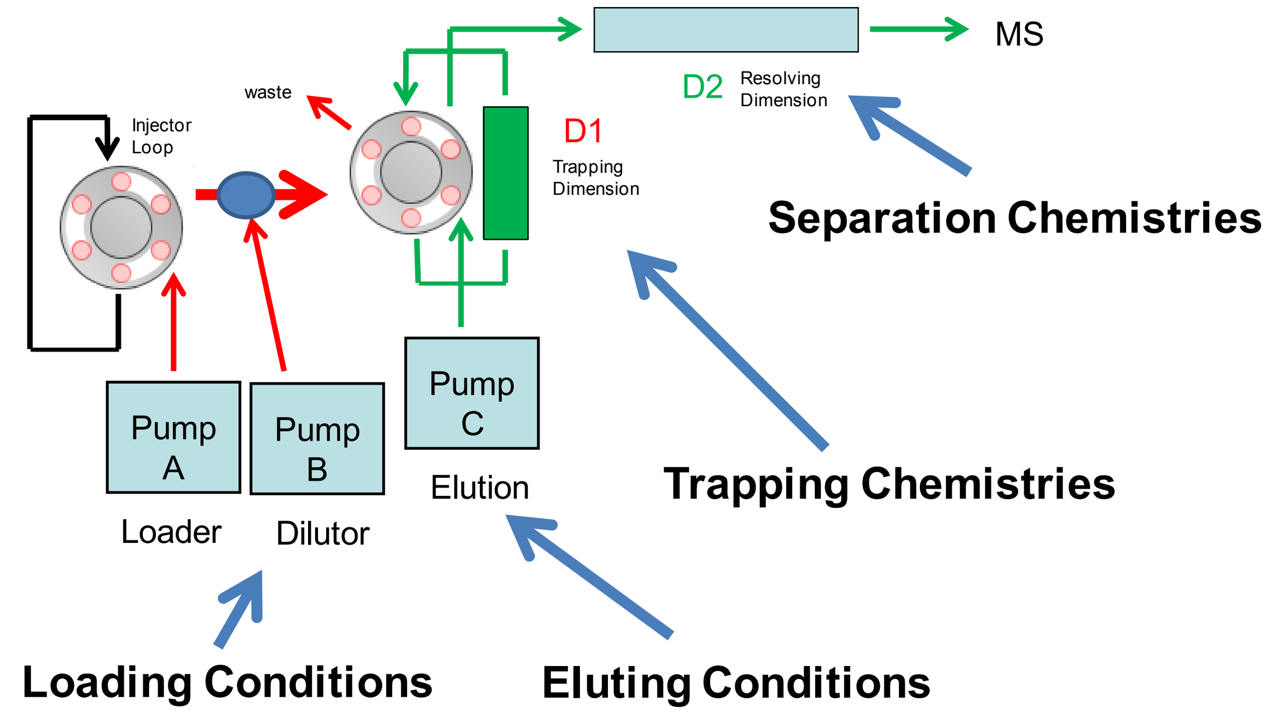
As seen in the past few decades, the reputations of many professional sports leagues and players have been tarnished due to cheating in the form of sports doping. As such, it is commonplace to have organizations, protocols and methods dedicated to detecting performance enhancing agents in biological samples such as urine. While doping commonly refers to steroids, the World Anti-Doping Agency (WADA)'s list of prohibited substances contains a wide variety of compounds with anabolic agents being only one of many classes. The wide variety of compound, low levels and the short time frame in which results are expected all contribute to the challenges of developing a comprehensive screening method for sports doping agents.

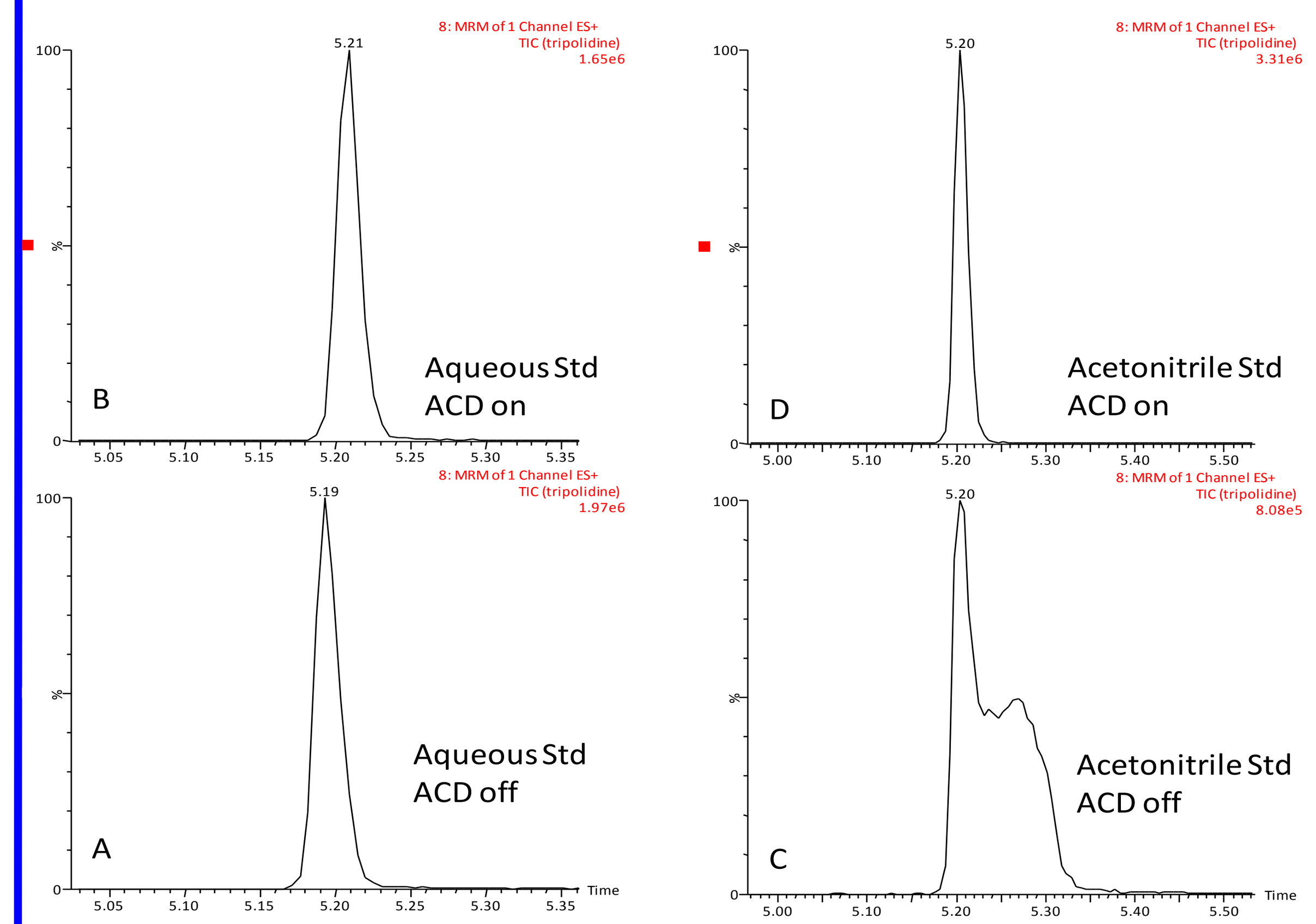
All target drugs in this screening study were classified in 9 classes based on their chemical structure (79 total). Once in solution, target analytes were individually infused to acquire their respective precursor and product ions. Once the quantitative and qualitative transitions for all drugs were identified, the chromatography conditions were optimized for each class using a 6x6 automated methods development protocol. Each target analyte was subjected to a total of 108 LC/MS/MS methods which were carried out within 72 hours. The multi-dimensional LC was configured for "Trap & Elute" with At-Column Dilution. The total run time was 10 minutes. The mass spectrometer was a XEVO G2 QToF operated in positive ionization mode.

Current analytical techniques use a combination of extraction procedures, often requiring an enrichment process and accurate detection for any given target analyte. As such, new analytical strategies are needed to reach those goals. This work evaluated the performance of 2D LC variant using a QToF setup instead of a triple quadrupole mass spectrometer for the analysis of drug of abuse in urine targeting low and sub ppb level. From a previous study[1] focusing on the analysis illicits drugs in urine by 2D LC/MS/MS, target LOD at 100 ppt was reported from a 1 mL urine sample. Considering the level of complexity of urine samples and also ensuring the retention of all target analyte in a clean extract, several strategic extraction protocols were evaluated to determine which one can reach a target detection level between 0.1 ppb and 1 ppb.

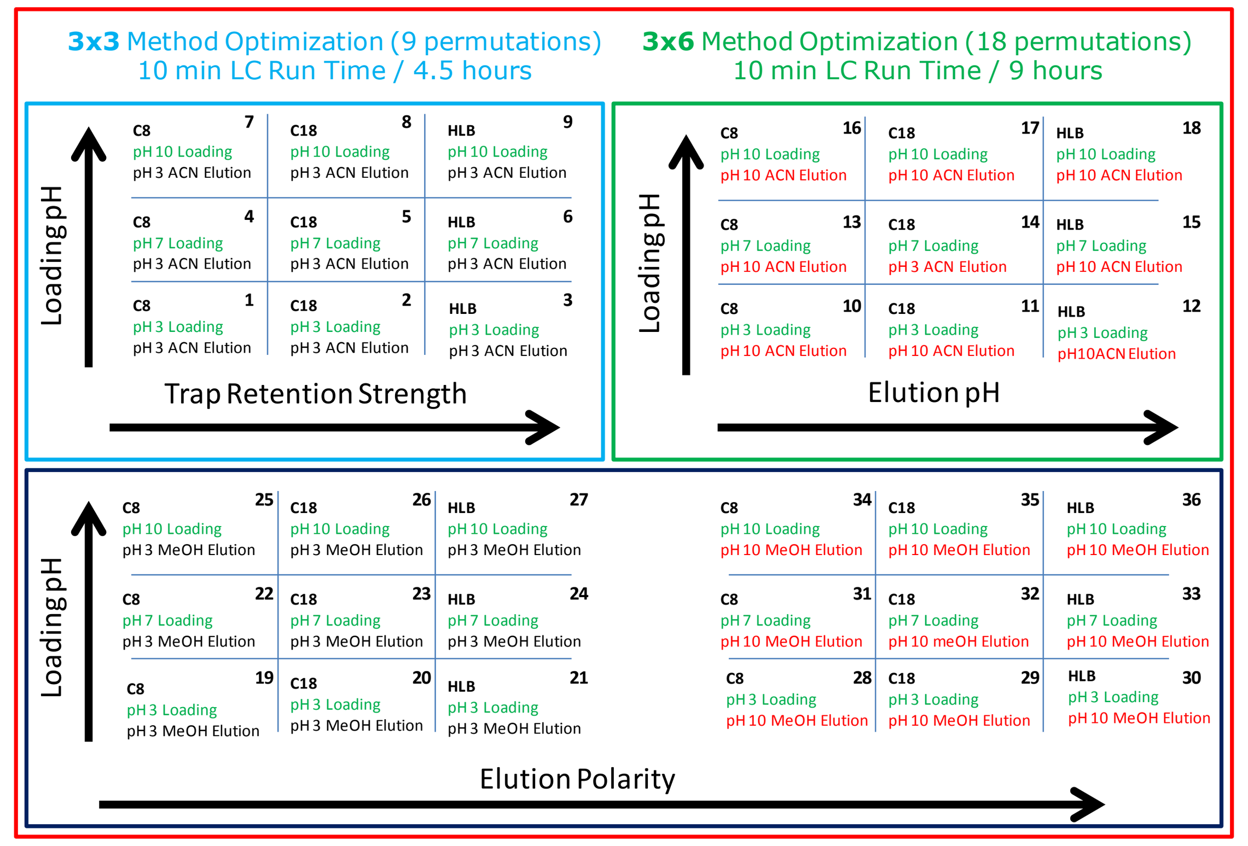
1-Mallet, C.R, Botch-Jones, S.R., Ilicit Drug Analysis Using Two-Dimension Liquid Chromatography/Tandem Mass Spectrometry, *J. Anal. Tox.*, 2016:1-11

INSTRUMENTATION





6x6 Method Optimization (36 permutations)
10 min LC Run Time / 18 hours

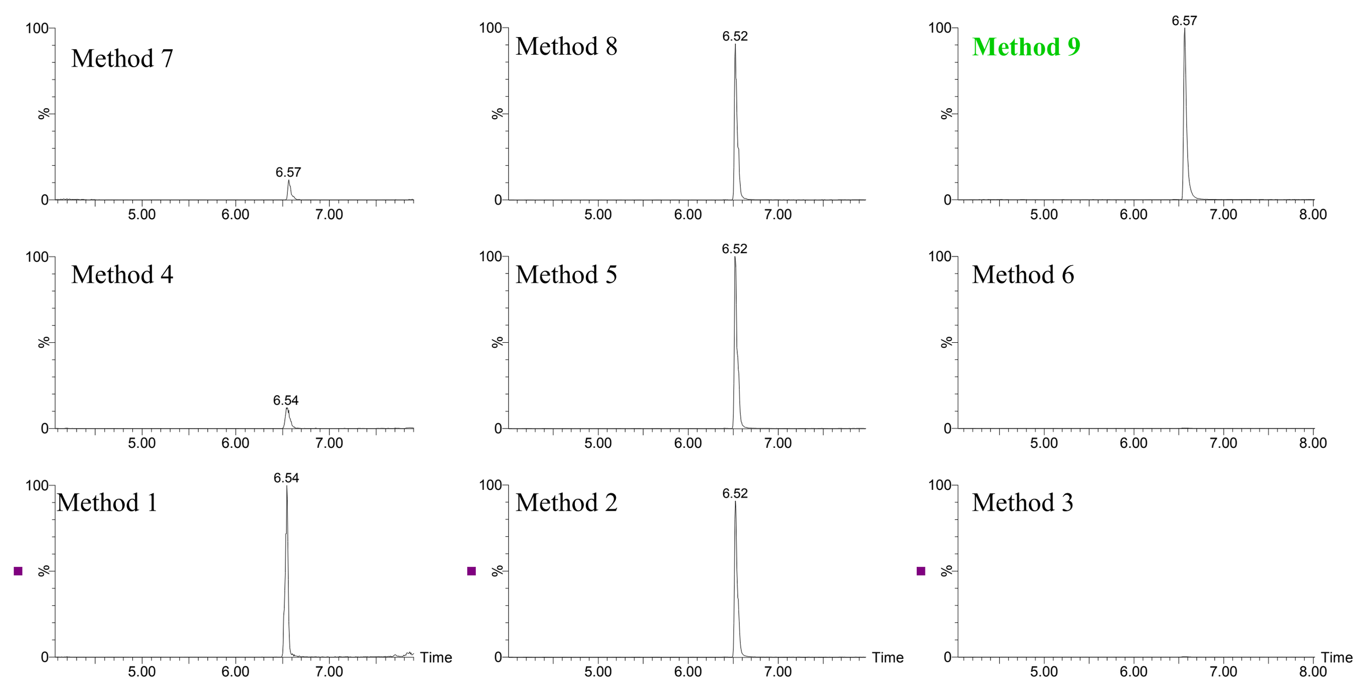


EXPERIMENTAL

Method	1	2	3	4	5	6	7	8	9
A - H2O									
A - MeoH	lead	e5	lead	e3 MP	e5	e4 MP			shoulder
A - ACN	lead	lead	lead	lead	e5	shoulder			shoulder
B - H2O	e5 MP	e6 MP	e4		tail	e4	e5	e5 MP	e8
B - MeoH	e5 MP	e6 MP	e5		lead	e5	e5	e5	e8
B - ACN	e5 MP	e6 MP	e5		lead	e5	e5	e5	e8
C- H2O		e5			e4			e4	e5
C - MeoH		e5			e4 MP				e5
C - ACN		e5			e4 MP			e4 MP	e5
D - H2O	e5	e6			e5			e5	e6
D - MeoH	e5	e7	e4	e5	e7	e4	e5	e7	e7
D - ACN	e5	e7	e4	e4	e7	e4	e4	e7	e7
E - H2O	e4 MP	e6 MP	e4		e5		e4 MP	e5	e6
E - MeoH	e5 MP	e6	e4	e4	e6	e4	e4 MP	e6	e7
E - ACN	e4 MP	e6	e4	e4	e6	e4	e4 MP	e6	e6
F - H2O									
F - MeoH	e4 MP	e6 MP	e4 MP	e4 MP	e6	e4 MP			
F - ACN	e4 MP	e6 MP	e4 MP	e4 MP	e6	e4 MP			
G - H2O	e4 MP	e5 MP	e4 MP		e5 MP				e5 MP
G - MeoH	e5 MP	e6	e5	e5	e6	e4	e4 MP	e5 MP	e6 MP
G - ACN	e5 MP	e6	e4	e4	e6	e4	e4 MP	e5 MP	e6
H - H2O	e5 MP	e7 MP	e4 MP	e4 MP	e6 MP	e4 MP	e4 MP	e6 MP	e7 MP
H - MeoH	e5 MP	e7 MP	e5 MP	e5 MP	e7 MP	e5 MP	e5 MP	e7 MP	e7 MP
H - ACN	e5 MP	e7 MP	e5 MP	e5 MP	e7 MP	tail	e5 MP	e7 MP	e7 MP

A- 5α-Dihydrotestosterone B- Clenbuterol C- Epitestosterone D- Danazol E- Androstenedione F- Tibolone G- Trans-Androsterone H- Testosterone

Danazol (MeOH)



Methods	1	2	3	4	5	6	7	8	9
Highest Signal	0	5	0	0	4	0	0	2	6
	10	11	12	13	14	15	16	17	18
	3	3	3	4	0	2	2	0	2
	19	20	21	22	23	24	25	26	27
	8	7	1	5	8	1	7	7	1
	28	29	30	31	32	33	34	35	36
	4	2	0	4	2	0	3	2	0

	Single Methods																					
Class 1	20	23																				
Class 2	1	2	3	6																		
Class 3	NA																					
Class 4	2	6																				
Class 5	8	9	17	24	25	27	35															
Class 6	8	9	15	26	35																	
Class 7	8	9	16	17	19	25	32	35														
Class 8	NA																					
Class 9	6	7	8	9	14	17	26	31	32													
Class 10	1	2	5	9	10	11	13	15	16	18	19	20	22	23	25	26	28	29	31	32	34	35

Multiple Methods: 9 19

Maximum Coverage for all 79 Pharmaceuticals Method 6, 9 and 19

Results

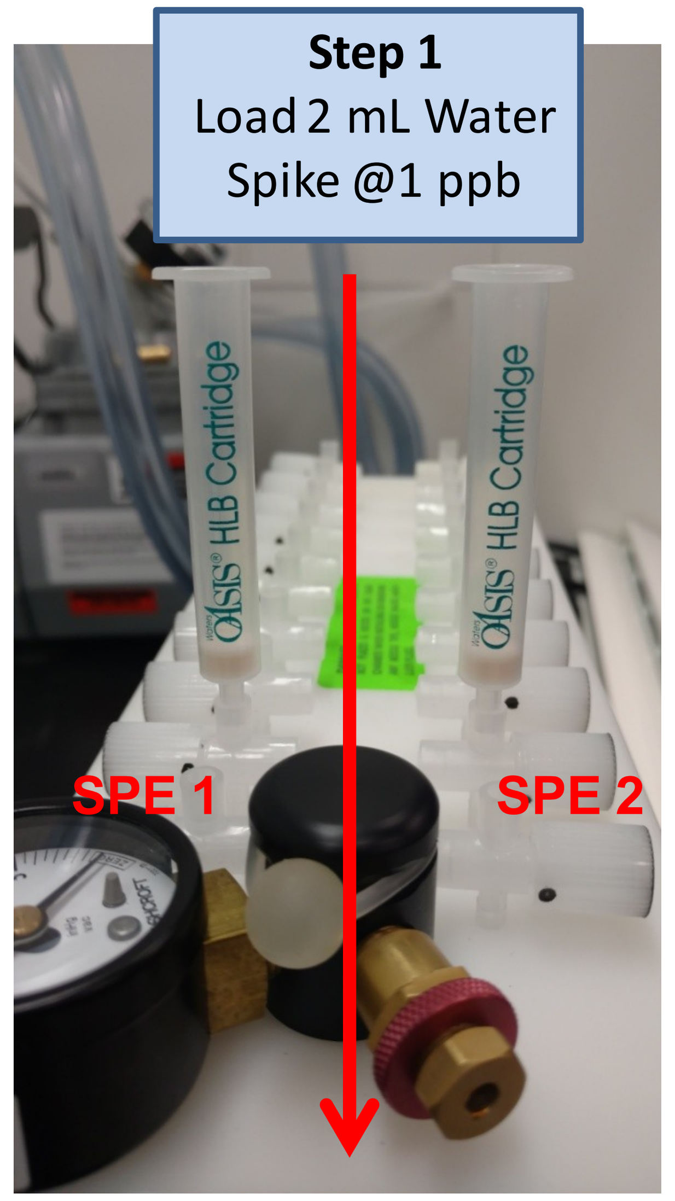
HLB 2D Protocol

Step 3 Sequential Elution

1- 10 % ACN
2- 20 % ACN
3- 30 % ACN
4- 40 % ACN
5- 50 % ACN
6- 60 % ACN
7- 70 % ACN
8- 80 % ACN
9- 90 % ACN
10- 100 % ACN

All elution with 2% FA

Repeat with MeOH



Step 4 Sequential Elution

1- 10 % ACN
2- 20 % ACN
3- 30 % ACN
4- 40 % ACN
5- 50 % ACN
6- 60 % ACN
7- 70 % ACN
8- 80 % ACN
9- 90 % ACN
10- 100 % ACN

All elution with 2% NH_4OH

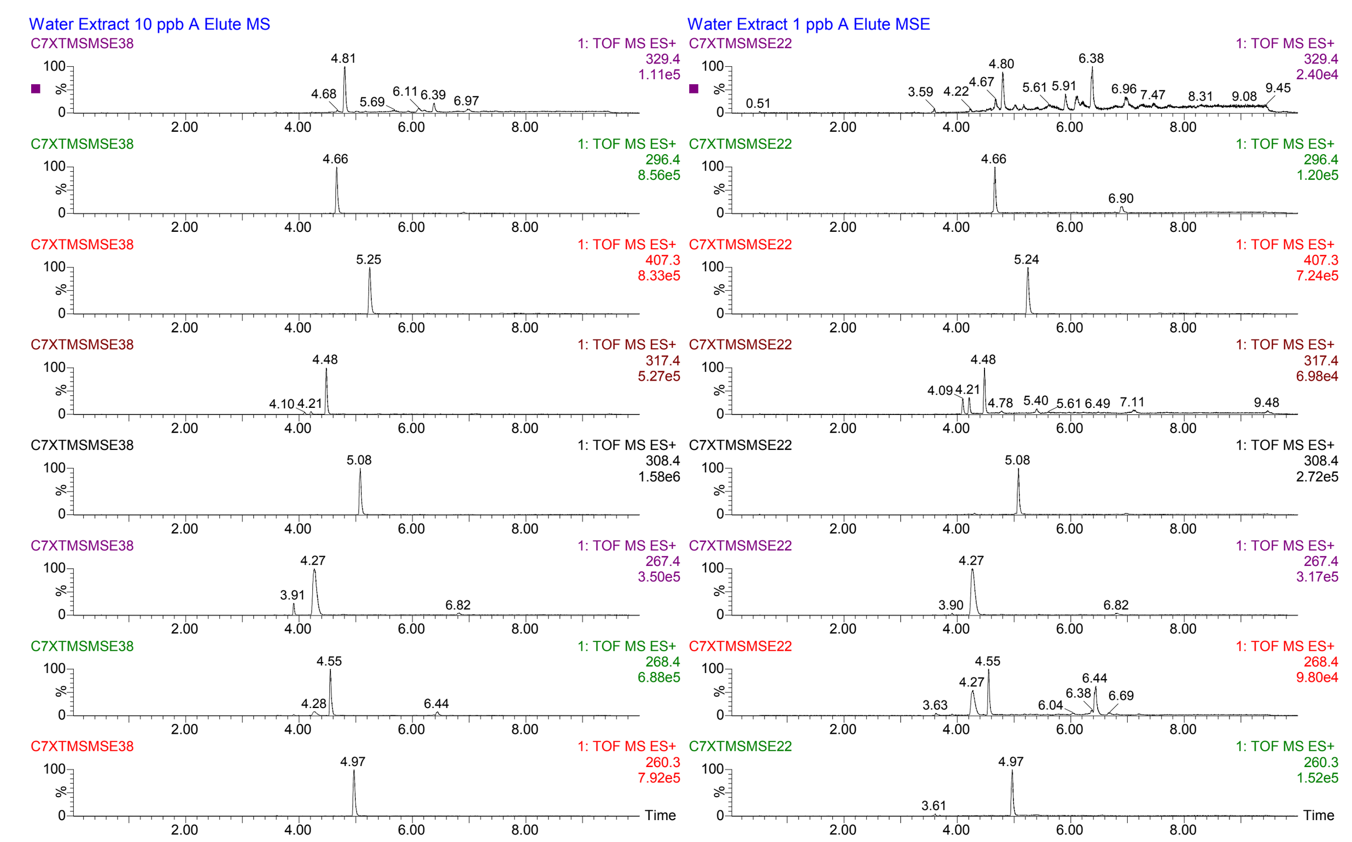
Repeat with MeOH

Class 7

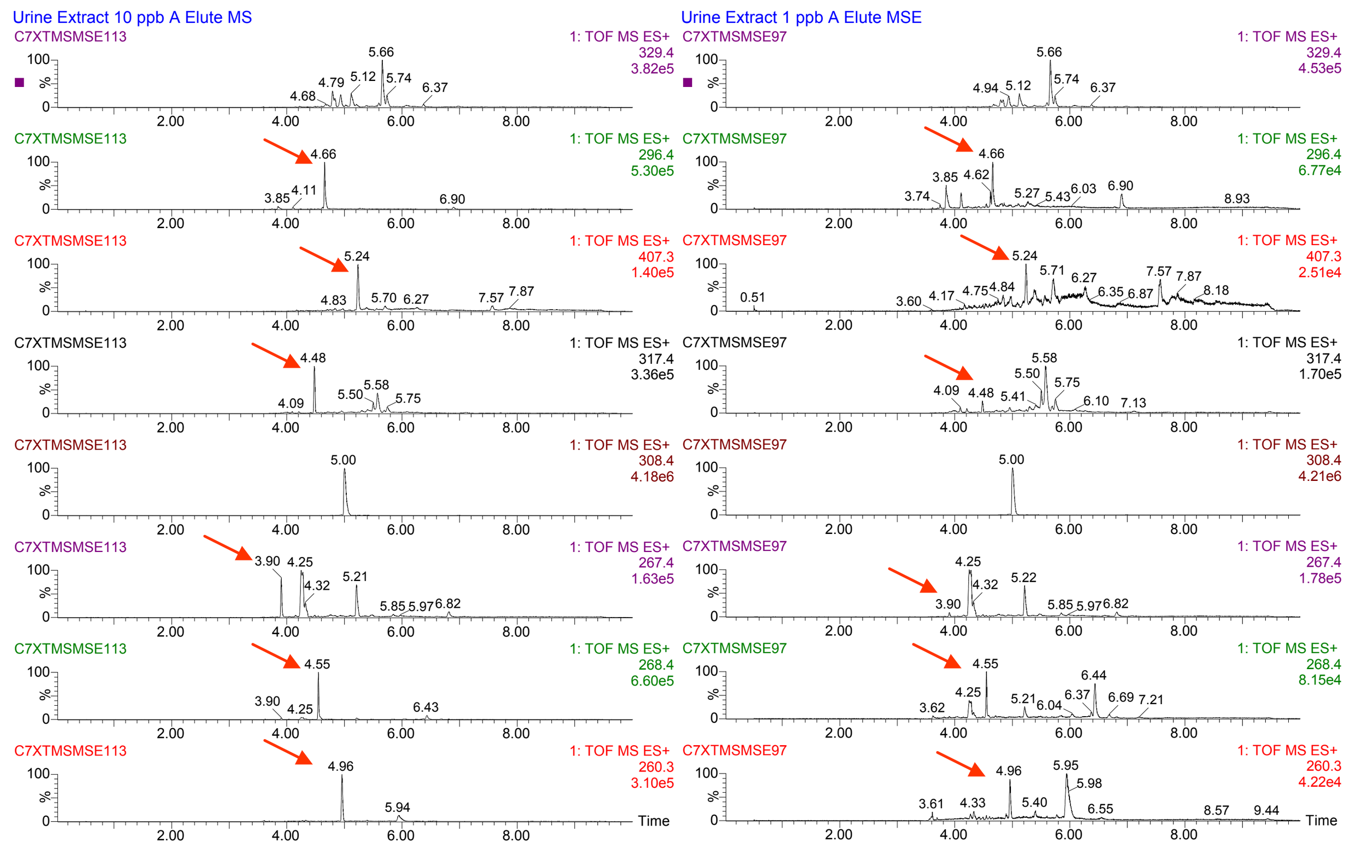
	0	10	20	30	40	50	60	70	80	90	100
Propanolol Acn Hi pH	0.09	0.00	1.42	1.04	55.06	37.47	3.37	1.01	0.05	0.27	0.2
Propanolol Acn Lo pH	0.75	0.08	74.23	23.04	0.54	1.01	0.05	0.03	0.18	0.04	0.06
Propanolol MeOH Hi pH	0.68	0.21	0.01	0.87	0.00	0.05	1.02	8.97	64.13	22.51	1.54
Propanolol MeOH lo pH	0.16	0.04	0.03	1.47	89.08	7.38	1.36	0.02	0.01	0.41	0.04
Atenolol Acn Hi pH	0.57	62.12	17.7	1.25	2.68	3.9	3.26	2.08	3.46	2.42	0.56
Atenolol Acn Lo pH	0.33	52.33	43.27	0.68	0.69	0.42	0.95	0.20	0.51	0.4	0.23
Atenolol MeOH Hi pH	1.19	0.30	0.57	93.8	0.87	0.75	0.71	0.65	0.47	0.49	0..4
Atenolol MeOH lo pH	0.62	88.26	6.26	0.69	1.01	1.54	0.23	0.36	0.26	0.43	0.19
Metaprolol Acn Hi pH	6.06	0.76	45.32	30.05	3.16	1.72	4.64	3.75	1.73	1.48	1.34
Metaprolol Acn Lo pH	1.54	40.21	54.46	1.68	0.43	0.28	0.05	0.71	0.11	0.02	0.54
Metaprolol MeOH Hi pH	0.2	0.00	0.58	60.35	5.1	13.8	7.52	10.34	0.11	0.95	1.05
Metaprolol MeOH lo pH	0.57	0.66	38.61	50.27	3.42	0.31	1.25	0.22	1.49	1.20	2.00
Esmolol Acn Hi pH	0.05	0.01	23.29	72.48	3.24	0.13	0.11	0.24	0.00	0.37	0.07
Esmolol Acn Lo pH	0.00	9.55	84.3	5.11	0.07	0.71	0.13	0.00	0.07	0.05	0.00
Esmololl MeOH Hi pH	0.03	0.00	0.02	25.42	1.07	9.48	14.79	38.97	8.47	1.67	0.10
Esmolol MeOH lo pH	0.00	0.03	2.41	79.54	17.38	0.08	0.02	0.00	0.47	0.00	0.08
Betaxolol Acn Hi pH	0.27	0.22	0.26	34.33	57.09	5.29	0.59	0.44	0.66	0.28	0.57
Betaxolol Acn Lo pH	0.21	0.12	86.23	10.74	1.48	0.36	0.53	0.11	0.00	0.11	0.12
Betaxolol MeOH Hi pH	0.37	0.02	0.19	0.79	0.22	0.94	1.9	23.58	58.97	11.81	1.21
Betaxolol MeOH lo pH	0.00	0.11	0.18	8.48	82.75	6.56	0.85	0.29	0.50	0	0.29
Timolol Acn Hi pH	0.58	0.08	55.58	32.61	4.89	0.93	0.76	0.25	1.49	2.55	0.27
Timolol Acn Lo pH	0.00	41.46	55.68	2.23	0.37	0.05	0.01	0.05	0.00	0.05	0.09
Timolol MeOH Hi pH	0.37	0.02	0.19	0.79	0.22	0.94	1.9	23.58	58.97	11.81	1.21
Timolol MeOH lo pH	0.01	0.11	50.88	46.56	1.44	0.31	0.03	0.12	0.23	0.27	0.04
Carvedilol Acn Hi pH	2.35	2.44	2.56	2.69	2.95	54.12	17.65	6.17	3.81	3.06	2.14
Carvedilol Acn Lo pH	1.83	1.62	2.18	66.85	13.97	3.97	2.61	1.46	2.15	1.77	1.58
Carvedilol MeOH Hi pH	2.11	2.33	2.06	2.21	1.6	1.82	2.6	1.52	4.97	47.58	31.19
Carvedilol MeOH lo pH	1.27	1.6	1.75	1.43	1.77	19.69	37.71	24.27	5.6	2.78	2.12
Labetalol Acn Hi pH	0.73	13.33	77.89	1.06	1.34	0.25	1.19	0.96	1.85	0.46	0.95
Labetalol Acn Lo pH	0.74	1.24	87.65	4.5	0.76	1.12	0.9	0.88	1.37	0.29	0.55
Labetalol MeOH Hi pH	0.00	1.57	0.56	90.82	1.09	1.34	1.96	0.54	0.96	0.89	0.27
LabetalolMeOH lo pH	1.26	0.71	0.03	8.76	81.26	3.26	1.36	1.01	0.81	0.74	0.8

Elution - 40 % ACN Lo pH
Wash - 20 % MeOH Hi pH

HLB 2D Protocol Water extracted standard



HLB 2D Protocol Urine extract



Conclusions

- Low level detection (ppb)
- Fast sample preparation
- Rapid method development
- High level of reproducibility and robustness
- Good precision & accuracy (< 5 %)

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