R. Kazlauskas

WADA list

National Measurement Institute (NMI), Australian Sports Drug Testing Laboratory (ASDTL)

Abstract

Since 2004 each new World Anti-Doping Agency (WADA) Prohibited List which is a document within the WADA Code and referenced by the International Standard for Laboratories (ISL) has included a considerable number of new substances. These new substances are slowly being included in the laboratories' repertoire but many still need work. A comprehensive summary which includes important issues such as - source of compound, excretion studies and projected status is presented.

Introduction

The World Anti-Doping Agency (WADA) has updated the WADA Prohibited List each year and each update contains new substances or classes of substances for which laboratories will eventually need to have validated analytical procedures. Usually there is a lag time before laboratories are able to implement procedures for the analysis of the new compounds and this can be due to issues such as:

- Availability of the substance either as a pure reference standard or as a pharmaceutical preparation. Not all of the substances on the list are available as pharmaceutical preparations and may originate from clandestine materials.
- Availability of information on the drugs metabolism. For some substances this can be obtained from the literature. If literature is not available studies need to be undertaken by the laboratory. For some compounds this may be more difficult and ethical issues may delay or prevent the studies.
- Availability of reference standards for any metabolites important for the drugs detection.
- Need to utilise new analytical techniques, some of which may require a considerable change to the laboratory procedures and purchase of expensive instrumentation and availability of expert staff.

- Ability and willingness of authorities to pay for the analytical work.

Further pressure will be placed on laboratories by WADA which will wish for the analysis of new substances to be implemented as rapidly as possible. This results in several new substances being introduced into the WADA Proficiency Testing program and can have fairly adverse affects on laboratories if they do not keep up to date. WADA will assist where possible to provide educational samples consisting of administration urines for some of the new compounds to allow laboratories to become adept at analysis of the compound and/or its metabolites but many will need to be studied by the laboratory as part of its research and development program.

The new classes and substances that have been added to the WADA Prohibited List are:

- **Aromatase inhibitors**
  - Formestane and testolactone
- **Selective estrogen receptor modulators**
  - Raloxifine and toremifine
- **Antiestrogenic substances**
  - Cyclofenil and fulvestrant
- **5α-reductase inhibitors**
  - Finasteride and dutasteride
- **Anabolic agents**
  - boldione, 4-hydroxy-19-nortestosterone, methyldienolone, methyltrienolone, methasterone (2α, 17α-dimethyl-5α-androstane-3-one-17β-ol), methyl-1-testosterone (17β-hydroxy-17α-methyl-5α-androst-1-en-3-one), methylnortestosterone (17β-hydroxy-17α-methylestr-4-en-3-one), prostanozol ([3,2-c]pyrazole-5α-etioallocholane-17β-tetrahydropyranol), tibolone, zilpaterol and norclostebol
- **Stimulants**
  - Famprofazone, benzylpiperazine, cyclazodone, fenbutrazate, fencamine, isometheptene, p-methylamphetamine, norfenefrine, octopamine, ortetamine, oxilofrine, phenpromethamine, sibutramine and tuaminoheptane.

The data for each substance is presented in tabular form under each class of compound.

Results and Discussion

AROMATASE INHIBITORS

Substance	Current Source	Method of detection	Metabolites	Ions used for detection #	Pure substance Available?	Excretion available?	Publication
Testolactone	China (ASDTL)	steroid screen GC/MS	4,5 dihydro and tetrahydro-testolactone	tetrahydro; 448, 343 RRT 0.99	Y	Y@	1
Formestane	NMI	steroid screen GC/MS	4-hydroxy-androstenedione; 4-hydroxytestosterone	518, 503, 520, 505 RRT 1.30	Y	Y@	2

SELECTIVE ESTROGEN RECEPTOR MODULATORS

Substance	Current Source	Method of detection	Metabolites	Ions used for detection	Pure substance Available?	Excretion available?	Publication
Toremefine	Roche, ASDTL	LC/MS/MS method	Parent and Hydroxy metabolites	406 > 72 for toremifene and 422>72 for hydroxy metabolite	Y	Y@	3
Raloxifene	Sigma, ASDTL	LC/MS/MS method	Parent	474>269; 474>112; and 474>85	Y@	Y	3

@ World Association of Anti-Doping Scientists (WAADS) Quality Assurance sample (WAADS QA)

RRT to D3-Testosterone

ANTIESTROGENIC SUBSTANCES

Substance	Current Source	Method of detection	Metabolites	Ions used for detection	Pure substance Available?	Excretion available?	Publication
Cyclofenil	Searle	Steroid screen GC/MS	Hydroxy metabolite	422, 512, 343	Y	Y*	4
Fulvestrant	Tocris Bioscience, ASDTL	LC/MS/MS method	See WAADS website, not investigated	NA	Y	N	5

NA = Not Available

5a-REDUCTASE INHIBITORS

Substance	Current Source	Method of detection	Metabolites	Ions used for detection	Pure substance Available?	Excretion available?	Publication
Finasteride	NMI (metabolite)	LC/MS/MS	Finasteride acid	403>335	Y	Y*	6, 7
Dutasteride	ASDTL	NA	Not detectable in urine	A/E decreased, 5a/5b-diols decreased in steroid profile	Y	Y	

* WADA Educational sample

Note that dutasteride is administered in low dose of 500ug and most of a dose is excreted as metabolites in the faeces. At steady state the elimination half-life is about 3 to 5 weeks (8).

ANABOLIC AGENTS

Substance	Current Source	Method of detection	Metabolites	Ions used for detection	Pure substance Available?	Excretion available?	Publication
1-androstenediol (5α-androst-1-ene-3β,17β-diol)	NMI	Same as 1-Testosterone			Y	N	
1-androstenedione (5α-androst-1-ene-3,17-dione	NMI	Same as 1-Testosterone			Y	N	
bolandiol (19-norandrostenediol)	NMI	Same as Nandrolone			Y	Y	
boldione (androsta-1,4-diene-3,17-dione)	NMI	Same as Boldenone			Y	N	
4-hydroxytestosterone (4,17β-dihydroxyandrost-4-en-3-one)	NMI	GC/MS Steroid screen	4-hydroxy-androstenedione	518, 503, 520, 505	Y	Y	2, 11
methasterone (2α, 17α-dimethyl-5α-androstane-3-one-17β-ol)	ASDTL	GC/MS steroid screen	Parent 3-hydroxy metabolite	143, 462, 447 143, 449, 464	Y@	Y@	9, 10, 12

ANABOLIC AGENTS (CONT.)

Substance	Current Source	Method of detection	Metabolites	Ions used for detection	Pure substance Available?	Excretion available?	Publication
methyldienolone (17β-hydroxy-17α-methylestra-4,9-dien-3-one)	NMI	GC/MS steroid screen, LC/MS/MS	Parent	GC/MS 430, 325, 285 RRT 1.14*	Y	Y	-
methyl-1-testosterone (17β-hydroxy-17α-methyl-5α-androst-1-en-3-one)	NMI	GC/MS steroid screen	17-methylandrost-1-ene-3α,17β-diol, parent, 17-epiM1T, MeT M1	143, 448, 419 RRT 0.96*	Y	Y	12
methylnortestosterone (17β-hydroxy-17α-methylestr-4-en-3-one)	Steraloids, NMI	GC/MS steroid screen	5α and 5β - 17methylnor-androstanediols	143, 436, 421, RRT 0.81, 0.88*	Y	Y@	12, 13
Methyltrienolone	PerkinElmer Life and Analytical Sciences, ASDTL	LC/MS/MS	Expect parent but not done	285>227, 285>198	Y@	N	14, 15
prostanozol ([3,2-c]pyrazole-5α-etioallocholane-17β-tetrahydropyranol)	Supplement, ASDTL	GC/MS and LC/MS/MS	Hydroxy metabolites	GC/MS 544, 254, 529 RRT 1.62; LC/MS/MS 329>81, 329>95	Y	Y@	9, 10

* relative to D3-Testosterone.

ANABOLIC AGENTS (CONT.)

Substance	Current Source	Method of detection	Metabolites	Ions used for detection	Pure substance Available?	Excretion available?	Publication
desoxymethyltestosterone (17α-methyl-5α-androst-2-en-17β-ol)	Extracted from supplement	GC/MS Steroid screen	parent and Hydroxymetabolites	143, 345, 270 (parent)	Y	Y (8)	10, 18
Tibolone	ASDTL	GC/MS	3β-hydroxytibolone, 3α-hydroxytibolone and the D4-isomer of tibolone	443, 353, 209	Y	Y	19, 12
18-methylnandrolone (18α-homo-17β-hydroxyestr-4-ene-3-one)	ASDTL	GC/MS steroid screen	18-methylnor-androsterone and 18-methylnor-etiocholanolone	405, 434, 419 RRT 0.84*	Y	Y@	13
4-hydroxy-nortestosterone (oxabolone)	NMI	GC/MS steroid screen	4-hydroxynor-androstenedione	504, 489, 416 RRT 1.18*	Y	Y	16, 17
Zilpaterol	NMI	GC/MS Steroid screen	Expect unchanged drug, not studied	308, 291, 405	Y@	Y	20
Norclostebol	NMI	GC/MS steroid screen	3-hydroxy and 3-hydroxy-4,5-dihydro metabolites	437, 452, 454 RRT 0.99*	Y	Y@	13

@ WAADS QA

* relative to D3-Testosterone.

STIMULANTS

Substance	Current Source	Method of detection	Metabolites	Ions used for detection	Pure substance Available?	Excretion available?	Publication
Fencamine	NMI synthesis	GC/MS for methamphetamine,	methamphetamine	Parent 293, 236, 162, RRT to DPA 2.69	Y	N	3
Cyclazodone	NMI synthesis	GC/MS stimulants screen, GC/MS steroids screen	Not known but expect mainly parent	118, 189, 216 RRT to DPA 1.45; TMS 360, 345, 178, RRT 0.29*	Y	N	3
Fenbutrazate	NMI synthesis	GC/MS stimulant screen	Not know but expect parent and hydrolysis product	367, 261, 190 RRT DPA 1.65; Hydrolysis product 221, 190, 56, RRT DPA 1.16	Y	N	
isometheptene	ASDTL	GC/MS stimulants screen	Parent and Hydroxymetabolite	Parent 141, 126, 58; Hydroxymentabolite 157, 140, 58	Y@	Y@	WAADS
Sibutramine	Abbott, ASDTL	GC/MS steroids, LC/MS/MS	desmethyl and hydroxylated metabolites	NA	Y@	Y@	23, 24, 25, 26
2-aminoheptane	Sigma, ASDTL (±, +, -)	GC/MS stimulants	Parent; have to start temperature gradient near solvent front for screening.	44, 100, 114	Y	N	3, 27
Famprofazone	NMI synthesis	GC/MS Stimulants screen	famprofazone, p-hydroxydesmethyl, amphetamine, methylamphetamine, ephedrines	NA	Y	N	21
Benzylpiperazine	NMI synthesis	GC/MS Stimulants screen	Parent	91, 134, 176; RRT to DPA 0.85	Y	N	22, 9

*RRT to Methyltestosterone

STIMULANTS (CONT.)

Substance	Current Source	Method of detection	Metabolites	Ions used for detection	Pure substance Available?	Excretion available?	Publication
p-methyl-amphetamine	NMI synthesis	GC/MS stimulants screen	Expect parent	44, 77, 91, 105, 148,134; RRT to DPA 0.50	Y	N	3
Norfenefrine	NMI synthesis	GC/MS for stimulants	Expect parent	44, 77, 91, 105, 148,134; RRT to DPA 0.507	Y@	N	3
Phenprometh-amine	ASDTL	GC/MS for stimulants	Expect parent	44, 77, 91, 105, 149; RRT to DPA 0.44	Y@	N	3
Ortetamine	ASDTL	NA	Expect parent		Y	N	
Oxilofrine	ASDTL	NA	Expect parent		Y@	N	
Octopamine	NMI	NA	Expect parent		Y	N	

@ WAADS QA

The compounds in the table listed as NMIA synthesis were all synthesised and made available to WADA accredited laboratories for purchase. There was no other source of these compounds available. This was funded in part by the Anti-Doping Research Panel.

Acknowledgements

Projects were supported by the World Anti-Doping Agency as well as by the Australian Government through the Anti-Doping Research program (ADRP) of the Department of Health and Aging.

References

World Anti-Doping Agency. The 2008 Prohibited List. International Standard, Montreal (2008) http://www.wada-ama.org/rtecontent/document/2008_List_En.pdf (access date 25.04.2008)

1. Vitoriano, B., de Boer, D., Manzoni, C., Ajenjo, A., dos Reys, L.J.A.L .Preliminary results of the implementation of some metabolites of the anabolic androgenic testolactone in the screening procedures. Recent advances in Doping Analysis (7), Proceedings of the Manfred Donike Workshop 17th Cologne Workshop on Dope Analysis 14th to 19th March 1999, 153-159

2. Parr, M.K., Opfermann, G., Schanzer, W., Analytical Properties of 4-hydroxysteroids and some Esters., Recent advances in Doping Analysis (12), Proceedings of the Manfred Donike Workshop 22nd Cologne Workshop on Dope Analysis 7th to 12th March 2004, 129-138.

3. R. Kazlauskas, Supplements and the WADA List, Recent advances in Doping Analysis (15), Proceedings of the Manfred Donike Workshop 25th Cologne Workshop on Dope Analysis, 25th February to 2nd March 2007, 31-40.

4. Mareck-Engelke,U., Sigmund,G., Opferman,G., Geyer,H. and Schanzer,W., Screening for Tamoxifen, Clomiphene and Cyclofenil in Doping Analysis., Recent advances in Doping Analysis (9), Proceedings of the Manfred Donike Workshop, 19th Cologne Workshop on Dope Analysis 18th to 23th March 2001, 53-61

5. Data provided by Paris Laboratory – private communication to WADA accredited laboratories.

6. Geyer, H. Gorius, I. Dreyer, N. Mareck, U., Thevis, M., Schänzer, W.: Investigation about the effects and the detection of finasteride. In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck (eds.) Recent advances in doping analysis (13). Sportverlag Strauß, Köln (2005) 479-482

7. Thevis M, Geyer H, Mareck U, Flenker U, Schänzer W: Doping control analysis of the 5a-reductase inhibitor finasteride: Determination of its influence on urinary steroid profiles and detection of its major urinary metabolite. Ther. Drug Monit. 29 (2007) 236-247

8. Martindale: The Complete Drug Reference, © Pharmaceutical Press 2004, electronic version.

9. Kazlauskas, R., Miscellaneous Projects in Sports Drug Testing at the National Measurement Institute, Australia, 2005., Recent advances in Doping Analysis (14), Proceedings of the Manfred Donike Workshop, 24th Cologne Workshop on Dope Analysis 4th to 9th June 2006, 129-140.

10. Rodchenkov, G., Sobolevsky, T. and Sizol, V., New Designer Anabolic Steroids from Internet., Recent advances in Doping Analysis (14), Proceedings of the Manfred Donike Workshop 24th Cologne Workshop on Dope Analysis 4th to 9th June 2006, 141-150.

11. Kohler, M. et al, Metabolism of 4 hydroxyandrostenedione and 4 hydroxytestosterone: Mass spectrometric identification of urinary metabolites.,
Steroids, 72 (2007) 278-286.

12. Parr, M.K., Opfermann, G., Schänzer, W.: Detection of new 17-alkylated anabolic steroids on WADA 2006 list. . In: Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U. (eds.) Recent advances in doping analysis (14). Sportverlag Strauß, Köln (2006) 249-258

13. Kazlauskas, R., Miscellaneous Projects 2004., Recent advances in Doping Analysis (13), Proceedings of the Manfred Donike Workshop 23rd Cologne Workshop on Dope Analysis 27th February to 4th March 2005, 137-146.

14. Thevis, M., Geyer, H., Mareck, U., Schänzer, W.: Screening for Synthetic Steroids in Human Urine by LC-ESI-MS/MS. In: Schänzer W, Geyer H, Gotzmann A, Mareck U (eds.) Recent advances in doping analysis (13). Sportverlag Strauß, Köln (2005) 147-155

15. Guddat, S., Schänzer, W., Wachsmuth, P., Thevis, M.: Doping control screening procedure for trenbolone analogues in human urine by LC-(APCI)-MS/MS. In: Schänzer, W., Geyer, H., Gotzmann, A., Mareck, U. (eds.) Recent advances in doping analysis (15). Sportverlag Strauß, Köln (2007) 397-400

16. Grosse, J., Lang, R., Muller, R.K., Thieme, D., and Wahl, A., Appearance of an oxabolone metabolite after administration of OTC 19-norsteroids – metabolite or artefact., Recent advances in Doping Analysis (8), Proceedings of the Manfred Donike Workshop 18th Cologne Workshop on Dope Analysis 20th to 25th February 2000, 227-232

17. Barbarulo, M.V. et al, Capillary GC-MS investigation of the metabolism and excretion of oxabolone in man., J. High Resolution. Chromatography, 18 (1995), 705-708).

18. Sekera, M.H., Ahrens, B.D., Chang, Y-C., Starcevic, B., Georgakopoulos, C., Catlin, D.H., 2005, Another designer steroid: discovery, synthesis, and detection of ‘madol’ in urine., Rapid Commun. Mass Spectrom., 19: 781-784

19. Marques, M.A.S., Pereira, H.M.G., de Olivera, M.C., Talhas, I.B. and Neto, F.R.A., Validation of the determination of tibolone in human urine and saliva using GC-MS for doping control and clinical studies., Recent advances in Doping Analysis (12), Proceedings of the Manfred Donike Workshop, 22nd Cologne Workshop on Dope Analysis 7th to 12th March 2004, 403-407.

20. Hoof, N.V. et al., Detection of Zilpaterol (Zilmax) in calf urine and faeces with liquid chromatography – tandem mass spectrometry., Analytica Chimica Acta, 529, (2005), 189-197.

21. Shin, Ho-Sang, Trennung von optischen Isomeren durch Gas-Chromatographie und die Chiralitat im Metabolismus von Pharmaka., Dissertation for Doktor der Sportwissenschaften, Institute fur Biochemie, Deutsche Sporthochsule Koln, supervisor Professor M. Donike, 1993.

22. Manzoni, C., de Boer, D., Calcada, M., Povoa, I. and dos Reys, L., Some Analytical Data Relevant for the Detection of 1 –Benzylpiperazine, a Pharmacological Alternative for Amphetamine., Recent Advances in Doping Analysis (9), Proceedings of the Manfred Donike Workshop, 19th Cologne Workshop on Dope Analysis, 18th to 23rd March 2001, 239-243.

23. Strano-Rossi, S., Colamonici, C., Botre, F., Detection of sibutramine administration : a gas chromatography/mass spectrometry study of the main urinary metabolites.Rapid Communications in Mass Spectrometry, 21, (2007), 79-88

24. Link, M., Hakala, KS., Wsol, V., Kostiainen, R., Ketola, RA., Metabolite profile of sibutramine in human urine: a liquid chromatography-electrospray ionization mass spectrometric study. Journal of Mass Spectrometry, 41, (2006), 1171-1178.

25. Sigmund, G., Thevis, M., Schiffer, A.K., Schänzer, W.: Determination of N-desmethyl- and N-bisdesmethyl metabolites of Sibutramine in doping control analysis using liquid chromatography-tandem mass spectrometry. In: Schänzer, W., Geyer,H., Gotzmann, A., Mareck, U. (eds.) Recent advances in doping analysis (14). Sportverlag Strauß, Köln (2006) 403-406

26. Thevis, M., Sigmund, S., Schiffer, A.K., Schänzer, W.: Determination of N-desmethyl- and N-bisdesmethyl metabolites of Sibutramine in doping control analysis using liquid chromatography-tandem mass spectrometry. Eur. J. Mass Spectrom. 12, (2006) 129–136

27. Thevis, M., Sigmund, G., Koch, A., Schänzer, W.: Determination of tuaminoheptane in doping control urine samples. Eur.J.Mass.Spectrom., 13 (2007) 213-221