

Supplementary Information (SI) for

In-sewer stability assessment of steroids and selective androgen receptor modulators

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2 Figures, 6 Tables and 3 equations;

Materials and methods

Lichrosolv grade methanol and analytical grade hydrochloric acid (32%) were purchased from Merck Pty Ltd (Highway Bayswater, VIC, AU) and 0.2 μm RC filters from Agilent (Mulgrave, VIC, AU). Ammonium fluoride was sourced from Sigma-Aldrich (Castle Hill, NSW, AU). Water was purified to 18.2 $\text{M}\Omega\text{ cm}^{-1}$ using a Milli-Q ultrapure water system and filtered using a 0.22 μm filter (Millipore, Bedford, MA, USA).

Andarine, clenbuterol (hydrochloride), ligandrol, stenabolic, and YK-11 were purchase from Cayman Chemical (Ann Arbor, MI, USA). D_3 -boldenone, boldenone glucuronide (K salt), boldenone M1, d_9 -clenbuterol (hydrochloride), clostebol M1, dehydrochlormethyltestosterone, dehydrochlormethyltestosterone M1, drostanolone M1, drostanolone M1 glucuronide (Na salt), fluoxymesterone M1, fluoxymesterone M2, mesterolone M1, metenolone M1, metandienone M1, metandienone M2, metandienone M3, methasterone, methasterone M1, methyl-1-testosterone, methyltestosterone M1, methyltestosterone M2, d_3 -methyltestosterone M2, 19-norandrosterone, d_4 -19-norandrosterone, 19-norandrosterone glucuronic acid (Na salt), 19-noretiocholanolone, d_4 -19-noretiocholanolone, 19-noretiocholanolone glucuronic acid (Na salt), norethandrolone M1, norethandrolone M2, norethandrolone M3, oxandrolone M1, stenabolic M2, stenabolic M6, stanozolol M1, stanozolol M1 glucuronide, androsterone, d_4 -androsterone, androsterone glucuronide, etiocholanolone, d_5 -etiocholanolone, trenbolone M1, 17α -methyltestosterone, oxandrolone, testosterone, and d_3 -epitestosterone were sourced from National Measurement Institute (North Ryde, NSW, AU). Nandrolone was supplied by British Pharmacopoeia Commission Laboratory (Teddington, Middlesex, UK). Sigma-Aldrich (Castle Hill, NSW, AU) provided boldenone, estrone, metandienone, progesterone and trenbolone.

Androstenedione, $^{13}\text{C}_3$ -androstenedione, and epitestosterone were sourced from Cerilliant (Round Rock, TX, USA). D_3 -Nandrolone and d_3 -testosterone were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Fluoxymesterone was supplied by LGC (Luckenwalde, Germany). Cardarine M1, cardarine M2, methasterone M1, metenolone, methylstenbolone, testolone, d_5 -stanozolol M1, d_5 -trenbolone, d_5 -trenbolone M1 were purchased from Toronto Research Chemicals Inc. (North York, ON, CA). Enobosarm was sourced from Selleck Chemicals Llc (Houston, TX, USA).

Instrument method (LC-MS/MS)

Optimised MS parameters: temperature 530 °C, curtain gas 30 psi, IonSpray voltage 4500 V and -4500 V, and Ion Source Gas 1 and 2, 80 psi.

Table S1. Optimised MS conditions of each MRM transition including ESI polarity, precursor and selected product ions, declustering potential (DP), entrance potential (EP), collision energy (CE), and cell exit potential (CXP).

Analyte	ESI polarity	Precursor Ion (<i>m/z</i>)	Selected Product Ion (<i>m/z</i>)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
Method A							
13C3-Androstenedione	+	290.0	112.0	90	10	30	17
	+	290.0	100.0	90	10	28	12
17a-Methyltestosterone	+	303.2	267.1	100	10	24	16
	+	303.2	227.1	100	10	27	14
	+	303.2	109.1	100	10	33	18
19-Norandrosterone	+	259.3	241.3	120	10	18	20
	+	277.3	241.4	50	10	19	14
	+	259.3	145.3	120	10	26	20
19-Noretiocholanolone	+	259.3	241.4	140	10	15	15
	+	259.3	145.3	140	10	27	17
	+	259.3	201.4	140	10	21	25
Andarine	+	442.2	400.1	130	10	21	25
	+	442.2	208.2	130	10	27	12
	+	442.2	190.2	130	10	31	11
Androstenedione	+	287.3	97.0	80	10	26	15
	+	287.3	109.1	80	10	29	15
	+	287.3	251.1	80	10	23	15
Androsterone	+	273.3	255.4	165	10	17	14
	+	273.3	147.3	165	10	27	17
	+	273.3	105.3	165	10	43	24
Boldenone	+	287.2	121.1	58	10	30	15
	+	287.2	135.1	58	10	20	22
	+	287.2	269.1	58	10	14	16
Boldenone M1	+	289.3	271.3	110	10	17	17
	+	289.3	187.3	110	10	25	23
	+	289.3	201.3	110	10	22	24

Analyte	ESI polarity	Precursor Ion (<i>m/z</i>)	Selected Product Ion (<i>m/z</i>)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
Clenbuterol	+	277.2	203.1	60	10	22	12
	+	277.2	259.2	60	10	14	15
	+	277.2	132.2	60	10	39	14
Clostebol M1	+	305.3	269.2	120	10	13	17
	+	305.3	287.2	120	10	12	19
	+	305.3	251.2	120	10	19	15
d3-Boldenone	+	289.9	272.0	180	10	15	16
	+	289.9	137.9	180	10	20	22
	+	289.9	120.9	180	10	30	16
d3-Epitestosterone	+	291.9	108.9	100	10	32	13
	+	291.9	96.9	100	10	28	15
d3-Methyltestosterone M2	+	273.9	191.9	140	10	23	28
	+	273.9	178.0	140	10	24	19
	+	273.9	111.9	140	10	24	25
d3-Nandrolone	+	277.9	241.9	100	10	24	13
	+	277.9	108.9	100	10	34	12
d3-Testosterone	+	291.9	108.9	90	10	35	14
	+	291.9	96.9	90	10	31	14
d4-19-Norandrosterone	+	262.9	245.0	150	10	18	15
	+	262.9	189.0	150	10	26	23
	+	262.9	144.9	150	10	27	20
d4-19-Noretiocholanolone	+	262.9	245.0	150	10	16	14
	+	262.9	205.0	150	10	22	12
	+	262.9	189.0	150	10	22	11
d4-Androsterone	+	277.0	259.0	140	10	18	15
	+	277.0	160.9	140	10	25	22
	+	277.0	146.9	140	10	25	17
d5-Etiocholanolone	+	277.9	260.0	125	10	15	16
	+	277.9	220.0	125	10	21	25
	+	277.9	203.9	125	10	21	26
d5-Stanozolol M1	+	350.2	97.0	210	11	52	22
	+	350.2	91.1	210	11	105	12
	+	350.2	77.1	210	11	105	12
d5-Trenbolone	+	276.2	258.1	125	10	28	15
	+	276.2	232.1	125	10	31	13

Analyte	ESI polarity	Precursor Ion (<i>m/z</i>)	Selected Product Ion (<i>m/z</i>)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
	+	276.2	204.1	125	10	30	11
d5-Trenbolone M1	+	276.2	258.1	150	10	28	16
	+	276.2	230.0	150	10	31	13
	+	276.2	204.1	150	10	32	12
d9-Clenbuterol	+	285.8	203.9	70	10	22	24
	+	285.8	132.9	70	10	40	19
	+	287.8	205.8	60	10	22	21
Dehydrochlormethyltestosterone	+	335.3	317.3	90	10	16	19
	+	317.2	155.2	165	10	32	20
	+	335.2	155.2	90	10	35	18
Dehydrochlormethyltestosterone M1	+	333.2	315.1	155	10	15	19
	+	351.3	315.1	50	10	15	18
	+	333.2	155.1	155	10	34	20
Drostanolone M1	+	287.4	269.4	136	10	17	17
	+	287.4	145.3	136	10	24	20
	+	287.4	121.3	136	10	27	20
Epitestosterone	+	289.3	97.3	80	10	30	12
	+	289.3	109.3	80	10	31	16
	+	289.3	253.3	80	10	24	15
Estrone	-	269.3	145.3	-125	-10	-47	-10
	-	269.3	143.3	-125	-10	-69	-16
	-	269.3	159.3	-125	-10	-46	-10
Etiocholanolone	+	273.2	255.2	100	10	15	16
	+	273.2	215.2	100	10	21	26
	+	273.2	199.2	100	10	21	24
Fluoxymesterone	+	337.4	241.3	80	10	33	15
	+	337.4	223.3	80	10	33	14
	+	337.4	181.3	80	10	40	18
Fluoxymesterone M1	+	337.4	95.1	125	10	27	15
	+	337.4	317.2	125	10	16	19
	+	337.4	299.1	125	10	19	20
Fluoxymesterone M2	+	319.3	281.1	130	10	25	15
	+	319.3	225.1	130	10	31	25
	+	319.3	299.2	130	10	25	20
Cardarine	+	454.1	257.0	185	10	40	14

Analyte	ESI polarity	Precursor Ion (<i>m/z</i>)	Selected Product Ion (<i>m/z</i>)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
	+	454.1	188.1	185	10	59	22
	+	454.1	172.1	185	10	90	19
Cardarine M1	+	486.1	257.2	160	10	40	16
	+	486.1	256.2	160	10	65	13
	+	486.1	188.2	160	10	65	12
Cardarine M2	+	470.2	256.9	45	10	30	24
	+	470.2	255.9	45	10	20	25
	+	470.2	187.9	45	10	64	22
Ligandrol	+	339.3	199.2	145	10	40	11
	+	339.3	170.2	145	10	71	10
	+	339.3	152.2	145	10	65	17
Mesterolone M1	+	287.3	269.3	145	10	17	15
	+	287.3	161.3	145	10	22	19
	+	287.3	147.3	145	10	22	18
Metandienone	+	301.3	149.3	58	10	21	18
	+	301.3	121.3	58	10	34	15
	+	301.3	173.3	58	10	23	23
Metandienone M1	+	283.3	121.3	150	10	29	18
	+	301.3	121.3	60	10	32	19
	+	301.3	149.4	60	10	21	18
Metandienone M2	+	299.2	281.1	85	10	12	20
	+	299.2	147.1	85	10	25	17
	+	299.2	121.1	85	10	28	19
Metandienone M3	+	269.3	105.3	85	10	34	11
	+	269.3	201.3	85	10	25	25
	+	269.3	161.4	85	10	29	21
Methasterone	+	319.4	283.4	155	10	21	18
	+	319.4	301.4	155	10	19	18
	+	319.4	229.4	155	10	26	20
Methasterone M1	+	285.4	175.3	130	10	23	11
	+	285.4	161.3	130	10	23	9
	+	285.4	91.3	130	10	65	14
Metenolone	+	303.4	187.3	115	10	28	11
	+	303.4	83.2	115	10	25	21
	+	303.4	205.3	115	10	23	12

Analyte	ESI polarity	Precursor Ion (<i>m/z</i>)	Selected Product Ion (<i>m/z</i>)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
Metenolone M1	+	285.2	267.2	145	10	17	15
	+	285.2	121.1	145	10	28	20
	+	285.2	119.1	145	10	35	15
Methyl-1-T	+	303.2	201.2	120	10	24	24
	+	303.2	145.1	120	10	34	20
	+	303.2	91.1	120	10	67	14
Methylstenbolone	+	317.4	201.3	130	10	25	12
	+	317.4	145.3	130	10	34	16
	+	317.4	91.2	130	10	70	10
Methyltestosterone M1	+	271.3	161.2	80	10	24	25
	+	271.3	147.2	80	10	24	21
	+	271.3	135.2	80	10	25	21
Methyltestosterone M2	+	271.2	175.1	125	10	25	26
	+	271.2	189.1	125	10	23	27
	+	271.2	109.1	125	10	26	24
Nandrolone	+	275.2	239.1	90	10	23	15
	+	275.2	109.1	90	10	34	17
	+	275.2	213.1	90	10	27	25
Norethandrolone M1	+	271.2	175.3	120	10	21	20
	+	271.2	147.3	120	10	21	18
	+	271.2	121.2	120	10	24	19
Norethandrolone M3	+	287.3	243.3	95	10	18	21
	+	287.3	147.3	95	10	28	17
	+	287.3	121.3	95	10	28	19
Enobosarm	+	407.3	390.1	60	10	16	23
	+	390.3	370.1	140	10	18	22
	+	390.3	187.1	140	10	18	22
Oxandrolone	+	307.2	271.1	50	10	18	15
	+	307.2	229.1	50	10	23	13
	+	307.2	253.1	50	10	21	14
Oxandrolone M1	+	307.3	289.4	80	10	14	17
	+	289.3	229.4	160	10	22	28
	+	289.3	135.3	160	10	26	22
Progesterone	+	315.2	97.1	105	10	26	15
	+	315.2	109.1	105	10	28	15

Analyte	ESI polarity	Precursor Ion (<i>m/z</i>)	Selected Product Ion (<i>m/z</i>)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
	+	315.2	79.0	105	10	69	12
Testolone	+	394.2	223.1	120	10	15	12
	+	396.2	225.1	115	10	15	13
	+	394.2	170.1	120	10	39	20
Stenabolic	+	438.2	125.2	100	10	30	13
	+	440.2	127.2	100	10	30	11
	+	438.2	89.2	100	10	100	40
Stenabolic M2	+	314.2	268.1	70	10	18	15
	+	314.2	142.0	70	10	26	18
	+	314.2	221.1	70	10	26	12
Stenabolic M6	+	283.3	125.1	60	10	18	20
	+	283.3	89.2	60	10	69	13
Stanozolol	+	329.1	107.1	100	10	51	16
	+	329.1	203.1	100	10	45	24
	+	329.1	121.1	100	10	47	14
Stanozolol M1	+	345.1	97.0	90	10	51	13
	+	345.1	97.0	260	10	51	12
	+	345.1	121.0	90	10	50	17
Stanozolol M1 gluc	+	521.0	345.0	90	10	32	20
	+	521.0	121.0	90	10	77	18
	+	521.0	97.0	90	10	80	15
Testosterone	+	289.3	97.3	92	10	27	17
	+	289.3	109.3	92	10	31	13
	+	289.3	123.3	92	10	32	14
Trenbolone	+	271.3	227.3	100	10	31	13
	+	271.3	199.3	160	10	46	6
	+	271.3	165.3	160	10	46	6
Trenbolone M1	+	271.3	253.3	155	10	28	14
	+	271.3	199.3	155	10	32	23
	+	271.3	165.3	155	10	70	20
YK-11	+	356.9	324.9	140	10	17	18
	+	357.0	307.1	140	10	26	18
	+	357.0	267.1	140	10	27	15
Method B							
19-Norandrosterone gluc	-	451.3	113.2	-140	-10	-35	-18

Analyte	ESI polarity	Precursor Ion (<i>m/z</i>)	Selected Product Ion (<i>m/z</i>)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
19-Noretiocholanolone gluc	-	451.3	273.3	-140	-10	-55	-24
	-	451.3	275.3	-140	-10	-40	-23
	-	451.3	113.2	-150	-10	-37	-13
	-	451.3	85.3	-150	-10	-36	-10
Andarine	-	451.3	273.3	-150	-10	-55	-16
	-	440.4	261.4	-105	-10	-25	-11
	-	440.4	150.5	-105	-10	-36	-21
Androsterone	-	440.4	107.4	-105	-10	-69	-17
	+	273.4	105.4	165	10	43	24
	+	273.4	147.4	165	10	27	17
Androsterone gluc	+	273.4	255.5	165	10	17	14
	-	465.3	113.3	-135	-10	-36	-13
	-	465.3	447.3	-135	-10	-30	-20
Boldenone gluc	-	465.3	157.3	-135	-10	-35	-15
	-	461.3	113.3	-160	-10	-34	-12
	-	461.3	85.3	-160	-10	-33	-11
Clenbuterol	-	461.3	157.2	-160	-10	-32	-18
	+	277.3	132.3	60	10	39	14
	+	277.3	203.2	60	10	22	12
d5-Etiocholanolone	+	277.3	259.3	60	10	14	15
	+	278	204	125	10	21	26
	+	278	220.1	125	10	21	25
d9-Clenbuterol	+	278	260.1	125	10	15	16
	+	285.9	133	70	10	40	19
	+	285.9	204	70	10	22	24
Drostanolone M1	+	287.9	205.9	60	10	22	21
	+	287.5	121.4	136	10	27	20
	+	287.5	145.4	136	10	24	20
Drostanolone M1 gluc	+	287.5	269.5	136	10	17	17
	-	479.5	113.2	-170	-10	-37	-13
	-	479.5	85.2	-170	-10	-36	-12
Etiocholanolone	-	479.5	157.2	-170	-10	-36	-14
	+	273.3	199.3	100	10	21	24
	+	273.3	215.3	100	10	21	26
Cardarine	+	273.3	255.3	100	10	15	16
	+	454.2	172.2	185	10	90	19
	+	454.2	188.2	185	10	59	22
Cardarine M1	+	454.2	257.1	185	10	40	14
	-	452.1	138.2	-75	-10	-30	-12
	-	452.1	394.1	-75	-10	-20	-21
Cardarine	-	452.1	123.2	-75	-10	-55	-19
	+	486.2	188.3	160	10	65	12

Analyte	ESI polarity	Precursor Ion (<i>m/z</i>)	Selected Product Ion (<i>m/z</i>)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
Cardarine M1	+	486.2	256.3	160	10	65	13
	+	486.2	257.3	160	10	40	16
	-	484.2	426.3	-65	-10	-22	-16
	-	484.2	170.3	-65	-10	-36	-21
Cardarine M2	-	484.2	122.4	-65	-10	-44	-18
	+	470.3	188	45	10	64	22
	+	470.3	256	45	10	20	25
Cardarine M2	+	470.3	257	45	10	30	24
	-	468.2	212.2	-45	-10	-19	-11
	-	468.2	154.2	-45	-10	-35	-16
Ligandrol	-	468.2	139.2	-45	-10	-40	-16
	-	337.4	267.4	-110	-10	-14	-12
	-	337.4	170.4	-110	-10	-35	-21
Metandienone M3	-	337.4	239.4	-110	-10	-25	-10
	+	269.1	213.2	85	10	22	13
	+	269.4	161.5	85	10	29	21
Methasterone M1	+	269.4	201.4	85	10	25	25
	+	285.5	91.4	130	10	65	14
	+	285.5	161.4	130	10	23	9
Methyltestosterone M1	+	285.5	175.4	130	10	23	11
	+	271.4	135.3	80	10	25	21
	+	271.4	147.3	80	10	24	21
Methyltestosterone M2	+	271.4	161.3	80	10	24	25
	+	271.3	109.2	125	10	26	24
	+	271.3	175.2	125	10	25	26
Norethandrolone M1	+	271.3	189.2	125	10	23	27
	+	271.3	121.3	120	10	24	19
	+	271.3	147.4	120	10	21	18
Norethandrolone M2	+	271.3	175.4	120	10	21	20
	+	271.3	121.2	70	10	21	20
	+	271.3	135.3	70	10	24	25
Testolone	+	289.3	271.3	115	10	13	17
	-	348.1	321.1	-90	-10	-13	-17
	-	348.1	127.3	-90	-10	-26	-16
Stenabolic M6	-	348.1	145.2	-90	-10	-15	-14
	+	283.4	89.3	60	10	69	13
	+	283.4	125.2	60	10	18	20
Stanozolol M1 gluc	-	519.4	343.3	-145	-10	-51	-18
	-	519.4	113.2	-145	-10	-31	-13
	-	519.4	175.3	-145	-10	-29	-14

Study design

Table S2. Experiment spiking schedule and level for anabolic agents, and isotopically labelled internal standards used during instrumental analysis.

Analyte	Spiking level (µg/L)	Time point spiked (N=3)									Isotopically labelled internal standard for LC analysis
		Week 1			Week 2			Week 3			
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	
Andarine	2				x	x	x				d ₅ -Trenbolone
Androstenedione	10		x	x				x			¹³ C ₃ -Androstenedione
Androsterone	13.3		x	x				x			d ₄ -Androsterone
Boldenone	5				x	x	x				d ₃ -Boldenone
Boldenone M1	10		x	x				x			d ₃ -Epitestosterone
Cardarine	2				x	x	x				d ₃ -Methyltestosterone M2
Cardarine M1	2							x	x	x	d ₅ -Trenbolone M1
Cardarine M2	10		x	x				x			d ₅ -Trenbolone
Clenbuterol	2				x	x	x				d ₉ -Clenbuterol
Clostebol M1	13.3		x	x				x			d ₅ -Etiocholanolone
Drostanolone M1	10		x	x				x			d ₃ -Methyltestosterone M2
Enobosarm	8				x	x	x				d ₃ -Testosterone
Epitestosterone	10		x	x				x			d ₃ -Epitestosterone
Estrone	8				x	x	x				¹³ C ₃ -Androstenedione
Etiocholanolone	13.3		x	x				x			d ₅ -Etiocholanolone
Fluoxymesterone	8				x	x	x				d ₃ -Boldenone
Fluoxymesterone M1	10		x	x				x			d ₅ -Trenbolone
Fluoxymesterone M2	10		x	x				x			d ₅ -Etiocholanolone
Ligandrol	5				x	x	x				d ₃ -Testosterone
Mesterolone M1	10		x	x				x			d ₃ -Methyltestosterone M2
Metandienone	5				x	x	x				d ₃ -Boldenone
Metandienone M1	10		x	x				x			d ₄ -19-Noretiocholanolone
Metandienone M2	10		x	x				x			d ₅ -Trenbolone
Metandienone M3	10		x	x				x			d ₃ -Methyltestosterone M2

Methasterone	8				x	x	x				d ₃ - Methyltestosterone M2
Methasterone M1	10	x	x	x							d ₃ - Methyltestosterone M2
Metenolone	5				x	x	x				d ₃ -Testosterone
Metenolone M1	13.3		x	x				x			d ₅ -Etiocholanolone
Methyl-1- testosterone	10				x	x	x				d ₃ -Testosterone
Methylstenbolone	5				x	x	x				d ₃ - Methyltestosterone M2
Methyltestosterone	10				x	x	x				d ₃ -Testosterone
Methyltestosterone M1	10		x	x				x			d ₃ - Methyltestosterone M2
Methyltestosterone M2	10		x	x				x			d ₃ - Methyltestosterone M2
Nandrolone	8				x	x	x				d ₃ -Nandrolone
19-Norandrosterone	10		x	x				x			d ₄ -19- Norandrosterone
19- Noretiocholanolone	13.3		x	x				x			d ₄ -19- Noretiocholanolone
Norethandrolone M1	10		x	x				x			d ₃ - Methyltestosterone M2
Norethandrolone M2	10		x	x				x			d ₃ - Methyltestosterone M2
Norethandrolone M3	10		x	x				x			d ₃ - Methyltestosterone M2
Oxandrolone	15				x	x	x				d ₃ -Testosterone
Oxandrolone M1	10		x	x				x			d ₃ -Epitestosterone
Progesterone	5				x	x	x				d ₃ -Testosterone
Stanozolol M1	10		x	x				x			d ₅ -Stanozolol M1
Stanozolol M1 gluc	5				x	x	x				d ₅ -Stanozolol M1
Stenabolic	5				x	x	x				d ₃ - Methyltestosterone M2
Stenabolic M2	10		x	x				x			d ₅ -Trenbolone
Stenabolic M6	10		x	x				x			d ₃ -Epitestosterone
Testolone	5				x	x	x				d ₃ -Boldenone
Testosterone	5				x	x	x				d ₃ -Testosterone
Trenbolone	10				x	x	x				d ₅ -Trenbolone
Trenbolone M1	10		x	x				x			d ₅ -Trenbolone M1

Dehydrochlormethyl -testosterone	8				x	x	x				d ₃ -Boldenone
Dehydrochlormethyl -testosterone M1	10		x	x				x			d ₅ -Trenbolone
YK-11	9				x	x	x				d ₃ -Testosterone
19-Norandrosterone gluc	10				x	x	x				d ₅ -Etiocholanolone
19-Noretiocholano lone gluc	10				x	x	x				d ₅ -Etiocholanolone
Androsterone gluc	15				x	x	x				d ₅ -Etiocholanolone
Boldenone gluc	5				x	x	x				d ₅ -Etiocholanolone
Drostanolone M1 gluc	8				x	x	x				d ₅ -Etiocholanolone
Stanozolol M1 gluc (method B)	5				x	x	x				d ₅ -Etiocholanolone

Results and discussion

Instrument performance assessment

Table S3. Instrument method performance assessment values including quantifier transition (MRM), ESI mode, LOD, LOQ, accuracy, precision, and linearity.

Analyte	MRM <i>m/z</i>	ESI	LOD ($\mu\text{g L}^{-1}$) <i>n</i> = 8	LOQ ($\mu\text{g L}^{-1}$) <i>n</i> = 8	%Accuracy \pm %RSD						Linearity		
					0.01 $\mu\text{g L}^{-1}$ <i>n</i> = 8	0.04 $\mu\text{g L}^{-1}$ <i>n</i> = 7	Low spike		0.63 $\mu\text{g L}^{-1}$ <i>n</i> = 8	1.25 $\mu\text{g L}^{-1}$ <i>n</i> = 8	Medium spike 5 $\mu\text{g L}^{-1}$ <i>n</i> = 8	High spike 10 $\mu\text{g L}^{-1}$ <i>n</i> = 8	(R^2) <i>N</i> \geq 5
Method A													
Andarine	442 \rightarrow 400	+	0.01	0.03	103 \pm 15	98 \pm 4	80 \pm 4				103 \pm 2	100 \pm 2	0.9986
Androstenedione	287 \rightarrow 97	+	0.03	0.08			95 \pm 11				103 \pm 2	102 \pm 2	0.9992
Androsterone	273 \rightarrow 255	+	0.43	1.29					114 \pm 9		103 \pm 3	96 \pm 4	0.9980
Boldenone	287 \rightarrow 121	+	0.01	0.03		98 \pm 8	99 \pm 4				101 \pm 2	101 \pm 2	0.9995
Boldenone M1	289 \rightarrow 187	+	0.13	0.38			109 \pm 11				99 \pm 3	102 \pm 4	0.9995
Cardarine	454 \rightarrow 257	+	0.02	0.05			68 \pm 9				103 \pm 4	94 \pm 3	0.9944
Cardarine M1	486 \rightarrow 257	+	0.01	0.03	105 \pm 6	104 \pm 4	76 \pm 4				108 \pm 2	104 \pm 2	0.9976
Cardarine M2	470 \rightarrow 257	+	0.01	0.02	110 \pm 5	106 \pm 3	75 \pm 3				103 \pm 3	101 \pm 2	0.9958
Clenbuterol	277 \rightarrow 203	+	0.01	0.02		96 \pm 5	90 \pm 3				105 \pm 1	103 \pm 1	0.9996
Clostebol M1	305 \rightarrow 269	+	0.54	1.63					116 \pm 11		102 \pm 5	99 \pm 5	0.9970
Drostanolone M1	287 \rightarrow 269	+	0.27	0.81					103 \pm 6		99 \pm 5	102 \pm 6	0.9997
Enobosarm	407 \rightarrow 390	+	0.05	0.15			100 \pm 5				100 \pm 3	100 \pm 4	0.9991
Epitestosterone	289 \rightarrow 97	+	0.03	0.10			109 \pm 11	99 \pm 7			102 \pm 2	100 \pm 2	0.9999
Estrone	269 \rightarrow 145	-	0.07	0.22				89 \pm 8			97 \pm 3	100 \pm 1	0.9979
Etiocholanolone	273 \rightarrow 255	+	0.11	0.32				69 \pm 15			103 \pm 7	101 \pm 3	0.9949
Fluoxymesterone	337 \rightarrow 241	+	0.12	0.35				97 \pm 12			106 \pm 4	102 \pm 3	0.9971
Fluoxymesterone M1	337 \rightarrow 95	+	0.02	0.05		90 \pm 6	97 \pm 6				98 \pm 5	101 \pm 6	0.9995
Fluoxymesterone M2	319 \rightarrow 281	+	0.02	0.05			86 \pm 7				102 \pm 2	102 \pm 2	0.9998

Analyte	MRM <i>m/z</i>	ESI	LOD	LOQ	%Accuracy ± %RSD						Linearity			
					Low spike						Medium spike	High spike	(R ²)	
					(µg L ⁻¹) n = 8	(µg L ⁻¹) n = 8	0.01 µg L ⁻¹ n = 8	0.04 µg L ⁻¹ n = 7	0.08 µg L ⁻¹ n = 8	0.31 µg L ⁻¹ n = 8	0.63 µg L ⁻¹ n = 8	1.25 µg L ⁻¹ n = 8	5 µg L ⁻¹ n = 8	10 µg L ⁻¹ n = 8
Ligandrol	339 → 199	+	0.01	0.03	98 ± 11	92 ± 4	93 ± 4					98 ± 1	100 ± 2	0.9989
Mesterolone M1	287 → 269	+	0.10	0.31				68 ± 14				99 ± 2	97 ± 1	0.9987
Metandienone	301 → 149	+	0.01	0.04		83 ± 5	93 ± 5					98 ± 1	100 ± 2	0.9984
Metandienone M1	301 → 149	+	0.02	0.07			72 ± 12					102 ± 3	100 ± 3	0.9994
Metandienone M2	299 → 281	+	0.004	0.01		114 ± 7	97 ± 9					100 ± 3	99 ± 1	0.9999
Metandienone M3	269 → 201	+	0.08	0.23				94 ± 8				99 ± 2	101 ± 2	0.9997
Methasterone	319 → 283	+	0.03	0.09			104 ± 11					95 ± 1	95 ± 2	0.9942
Methasterone M1	285 → 175	+	0.12	0.35				97 ± 12				97 ± 3	97 ± 1	0.9994
Metenolone	303 → 187	+	0.02	0.05			91 ± 7					102 ± 2	101 ± 3	0.9997
Metenolone M1	285 → 121	+	1.57	4.75								105 ± 9	106 ± 5	0.9924
Methyl-1-testosterone	303 → 201	+	0.01	0.03		101 ± 6	104 ± 3					98 ± 1	99 ± 1	0.9997
Methylstenbolone	317 → 201	+	0.005	0.014		104 ± 6	104 ± 2					96 ± 1	97 ± 1	0.9998
Methyltestosterone	303 → 109	+	0.02	0.05			88 ± 7					96 ± 2	99 ± 1	0.9992
Methyltestosterone M1	271 → 161	+	0.06	0.19				95 ± 6				97 ± 2	98 ± 1	0.9991
Methyltestosterone M2	271 → 175	+	0.59	1.79						100 ± 14		99 ± 5	97 ± 3	0.9963
Nandrolone	275 → 109	+	0.02	0.06			101 ± 7					103 ± 2	103 ± 2	0.9997
19-Norandrosterone	259 → 241	+	0.21	0.63						106 ± 5		105 ± 4	104 ± 4	0.9993
19-Noretiocholanolone	259 → 241	+	0.67	2.04								100 ± 4	100 ± 4	0.9958
Norethandrolone M1	271 → 175	+	0.15	0.44				111 ± 13				97 ± 1	98 ± 1	0.9995
Norethandrolone M2	271 → 135	+	0.08	0.25				80 ± 10				95 ± 3	98 ± 3	0.9999
Norethandrolone M3	287 → 243	+	0.08	0.24				71 ± 11				110 ± 3	104 ± 2	0.9930
Oxandrolone	307 → 271	+	0.05	0.16				99 ± 5				103 ± 3	103 ± 3	0.9992
Oxandrolone M1	289 → 229	+	0.11	0.32				75 ± 14				109 ± 4	106 ± 3	0.9990
Progesterone	315 → 97	+	0.01	0.02		102 ± 6	73 ± 4					100 ± 1	100 ± 2	0.9989

Analyte	MRM <i>m/z</i>	ESI	LOD	LOQ	%Accuracy ± %RSD						Linearity		
					Low spike						Medium spike	High spike	(R ²)
			(µg L ⁻¹) n = 8	(µg L ⁻¹) n = 8	0.01 µg L ⁻¹ n = 8	0.04 µg L ⁻¹ n = 7	0.08 µg L ⁻¹ n = 8	0.31 µg L ⁻¹ n = 8	0.63 µg L ⁻¹ n = 8	1.25 µg L ⁻¹ n = 8	5 µg L ⁻¹ n = 8	10 µg L ⁻¹ n = 8	N ≥ 5
Stanozolol M1	345 → 97	+	0.03	0.10			117 ± 11	104 ± 6			99 ± 1	98 ± 1	0.9998
Stanozolol M1 gluc	521 → 345	+	0.06	0.19				112 ± 5			109 ± 2	107 ± 3	0.9971
Stenabolic	438 → 125	+	0.01	0.03	95 ± 4	105 ± 5	78 ± 4				92 ± 2	97 ± 2	0.9970
Stenabolic M2	314 → 268	+	0.01	0.04	88 ± 21	107 ± 2	102 ± 5				96 ± 1	98 ± 2	0.9994
Stenabolic M6	283 → 125	+	0.05	0.14				94 ± 5			101 ± 2	102 ± 2	0.9974
Testolone	394 → 223	+	0.02	0.07			87 ± 10				100 ± 6	98 ± 4	0.9980
Testosterone	289 → 97	+	0.01	0.03		116 ± 4	94 ± 4				102 ± 1	102 ± 1	0.9999
Trenbolone	271 → 227	+	0.04	0.12			75 ± 21	107 ± 7			97 ± 2	98 ± 3	0.9992
Trenbolone M1	271 → 253	+	0.04	0.12			79 ± 19	103 ± 4			102 ± 1	102 ± 1	0.9992
DHCMT	317 → 155	+	0.01	0.02		99 ± 14	98 ± 4				98 ± 1	98 ± 2	0.9999
DHCMT M1	333 → 155	+	0.20	0.59					102 ± 5		99 ± 4	98 ± 2	0.9987
Method B													
19-Norandrosterone gluc	451 → 113	-	0.64	1.93							104 ± 4	106 ± 4	0.9988
19-Noretiocholanolone gluc	451 → 113	-	0.23	0.68					102 ± 11		101 ± 5	104 ± 4	0.9978
Andarine	440 → 261	-	0.01	0.02			110 ± 3				106 ± 4	103 ± 7	0.9970
Androsterone	273 → 255	+	1.48	4.47							91 ± 10	95 ± 9	0.9633
Androsterone gluc	465 → 113	-	0.57	1.73							99 ± 4	98 ± 4	0.9951
Boldenone gluc	461 → 113	-	0.07	0.20					106 ± 3		99 ± 3	100 ± 3	0.9979
Cardarine	454 → 257	+	0.01	0.04			113 ± 4				100 ± 3	94 ± 3	0.9866
Cardarine	452 → 138	-	0.01	0.02			118 ± 2				100 ± 3	96 ± 2	0.9929
Cardarine M1	486 → 257	+	0.02	0.06			108 ± 7				98 ± 1	98 ± 3	0.9974
Cardarine M1	484 → 426	-	0.02	0.06			116 ± 7				98 ± 3	99 ± 3	0.9988
Cardarine M2	470 → 256	+	0.02	0.06			115 ± 6				99 ± 2	98 ± 2	0.9978
Cardarine M2	468 → 212	-	0.01	0.03			112 ± 4				100 ± 2	99 ± 3	0.9986

Analyte	MRM <i>m/z</i>	ESI	LOD	LOQ	%Accuracy ± %RSD						Linearity		
					Low spike				Medium spike	High spike	(R ²)		
			(µg L ⁻¹) n = 8	(µg L ⁻¹) n = 8	0.01 µg L ⁻¹ n = 8	0.04 µg L ⁻¹ n = 7	0.08 µg L ⁻¹ n = 8	0.31 µg L ⁻¹ n = 8	0.63 µg L ⁻¹ n = 8	1.25 µg L ⁻¹ n = 8	5 µg L ⁻¹ n = 8	10 µg L ⁻¹ n = 8	N ≥ 5
Clenbuterol	277 → 203	+	0.01	0.03			100 ± 4				98 ± 2	99 ± 2	0.9983
Drostanolone M1	287 → 269	+	0.24	0.72					119 ± 10		99 ± 3	94 ± 5	0.9957
Drostanolone M1 gluc	479 → 113	-	0.62	1.88						82 ± 18	93 ± 7	99 ± 6	0.9969
Etiocholanolone	273 → 255	+	0.25	0.77					83 ± 15		98 ± 2	101 ± 3	0.9974
Ligandrol	337 → 267	-	0.12	0.37					105 ± 6		97 ± 7	100 ± 4	0.9980
Metandienone M3	269 → 213	+	0.11	0.35					103 ± 5		99 ± 2	98 ± 3	0.9985
Methasterone M1	285 → 161	+	0.26	0.78					110 ± 11		100 ± 2	97 ± 2	0.9979
Methyltestosterone M1	271 → 135	+	0.10	0.29					99 ± 6		97 ± 2	98 ± 2	0.9988
Methyltestosterone M2	271 → 109	+	0.44	1.32						105 ± 10	100 ± 4	98 ± 3	0.9952
Norethandrolone M1	271 → 175	+	0.11	0.33					104 ± 5		98 ± 2	96 ± 3	0.9988
Norethandrolone M2	271 → 135	+	0.17	0.51					102 ± 8		98 ± 2	97 ± 3	0.9975
Stenabolic M6	283 → 125	+	0.05	0.15			114 ± 16		102 ± 5		102 ± 5	99 ± 3	0.9967
Stanozolol M1 gluc	519 → 343	-	0.08	0.24					106 ± 4		101 ± 5	102 ± 5	0.9981
Testolone	348 → 321	-	0.05	0.16					95 ± 3		98 ± 3	100 ± 2	0.9992

Linearity (R^2) for all analytes in acidified and filtered wastewater was >0.995 , with the exception of cardarine (method A: 0.994 & method B: 0.986 for positive ionisation; 0.992 for negative ionisation), methasterone (0.994), metenolone M1 (0.992), norethandrolone M3 (0.993), etiocholanolone (method A: 0.994), and androsterone (0.963). Limits of detection and limits of quantification ranged from 0.004 – 1.56 $\mu\text{g/L}$ and 0.01 – 4.75 $\mu\text{g/L}$, respectively. Accuracies were 68 – 119%, and the precision (%RSD) range was 1 – 21% across all spiking levels and both methods. Data for each analyte can be found in Table S3.

Both LC methods demonstrated satisfactory performance and applicability for the detection and quantification of 59 anabolic agents in wastewater influent. Only cardarine showed $<70\%$ accuracy (68%) near the LOQ (method A), whereas all other compounds had satisfactory accuracies of 70 – 130% at or near their LOQs. At low, medium and high concentration levels, all analytes had RSD values below 20%, with the exception of trenbolone and stenabolic M2 (method A) which had an RSD of 21% at the low spike. The performance of the instrument methods was evaluated before the in-sewer samples were analysed, and it was concluded that both LC-MS/MS methods were suitable for the intended purpose. It should be noted that slightly different results may be obtained if this experiment was repeated with wastewater from a different WWTP, as background noise and interferences may differ.

Typical parameters of the feeding raw sewage¹

The real sewage is weekly collected from a pump station in Brisbane (Australia). Wastewater in this residential area is the typical domestic sewage with pH 7.5, low sulfide (<3 mgS/L), 10 – 30 mgS/L sulfate, low methane (<5 mgCOD/L), 180 – 200 mg/L SCOD with 50 mgCOD/L acetate and 10 – 20 mgCOD/L propionate as the major VFAs contents, 200 – 400 mg/L TSS, and 180 – 380 mg/L VSS. The collected fresh sewage is stored in a cold room under 4°C to minimise biological reactions. The feeding sewage is heated by a thermoregulator in a water bath to room temperature before entering the reactor.

Statistical analysis

Equations applied are as follows:

For zero order kinetics (eq1):

$$\frac{C_t}{C_0} = -k_0 \times t_i + e$$

For first order kinetics (eq2):

$$\frac{C_t}{C_0} = f_f \times e^{-k_f t_i}$$

For two-phase kinetics (eq3):

$$\frac{C_t}{C_0} = f_f \times e^{-k_f t_i} + f_s \times e^{-k_s t_i}$$

Parameters:

C_t : concentration at time t

C_0 : concentration at time zero

k_0 : the rate of degradation for zero order degradation

e: intercept of the zero-order degradation

f_f : the fraction of compound degraded during the fast reaction

k_f : the rate of fast degradation

f_s : the fraction of compound degraded during the slow reaction

k_s : the rate of slow degradation

t_i : time

In equations eq1, eq2, and eq3 the fraction C_t/C_0 was replaced by 90, 50, and 10 for 10, 50, and 90% reduction, respectively. During the next step those equations were solved for t_i in order to estimate the transformation times.

Quality assurance and control

Mean recoveries (n=4) ranged from 89-118%, exceptions being drostanolone M1 (141%), drostanolone M1 gluc (134%), fluoxymesterone M1 (42%), fluoxymesterone M2 (121%), mesterolone M1 (122%), metandienone M3 (169%), methyltestosterone M2 (125%), androsterone (123%), etiocholanolone (137%), cardarine M1 (143%), cardarine M2 (166%), and YK-11 (133%), and the mean duplicate differences ranged from 0.8-14.5% across all biomarkers (Table S4).

Table S4. QAQC results for each biomarker. Recovery mean % was calculated by subtracting the concentration of the analyte in the wastewater sample from the concentration of the spiked sample and dividing that by the non-extracted side spike. QC accuracy is the accuracy of the 10 µg/L calibration solution reinjected during the run compared to the calibration curve.

Analyte	Recovery mean % ±SD (n=4)	Duplicate difference mean % (n=7)	QC Accuracy %
Boldenone	110±11	1.4	101
Boldenone gluc	101±4	3.1 (n=3)	95
Boldenone M1	104±10	3.4	101
Clostebol M1	103±10	3.3	99
Drostanolone M1	141±8	6.4	107
Drostanolone M1 gluc	134±11	14.0 (n=5)	79
Estrone	104±12	3.8	95
Fluoxymesterone	100±13	6.8	91
Fluoxymesterone M1	42±7	5.5	115
Fluoxymesterone M2	121±8	4.3	107
Mesterolone M1	122±13	3.4	103
Methyl-1-testosterone	109±9	3.5 (n=6)	105
Metandienone	115±6	3.8	101
Metandienone M1	134±18	3.0	105
Metandienone M2	93±6	2.3	100
Metandienone M3	169±34	7.6	106
Methasterone	107±6	3.6	109
Methasterone M1	118±11	6.1 (n=5)	104
Metenolone	112±9	5.2	98
Metenolone M1	103±8	3.2	102
Methylstenbolone	110±4	4.7	106
Methyltestosterone	103±8	2.8	99

Methyltestosterone M1/Norethandrolone M2	116±12	2.7	107
Methyltestosterone M2	125±14	4.0	103
Nandrolone	102±7	2.1	100
19-Norandrosterone	104±12	4.2	106
19-Norandrosterone gluc	108±8	5.7 (n=4)	97
19-Noretiocholanolone	106±12	4.1	105
19-Noretiocholanolone gluc	105±6	1.7 (n=4)	95
Norethandrolone M1	115±11	3.6	108
Norethandrolone M3	107±8	4.0	99
Progesterone	117±10	4.6 (n=5)	100
Oxandrolone	111±31	7.5	82
Oxandrolone M1	117±18	6.5	102
Testosterone	105±7	0.8	101
Androstenedione	102±4	4.0 (n=6)	106
Androsterone	123±6	7.6	97
Androsterone gluc	108±13	3.6 (n=3)	95
Epitestosterone	109±11	5.6	101
Etiocholanolone	137±30	5.0	101
Stanozolol M1	92±17	2.0	101
Stanozolol M1 gluc	116±8	5.7 (n=4)	100
Trenbolone	114±11	2.4	105
Trenbolone M1	101±16	3.2	98
DHCMT	113±16	3.1	98
DHCMT M1	95±7	2.4	100
Andarine	106±5	4.2	92
Cardarine	96±28	7.8	112
Cardarine M1	143±23	14.5	105
Cardarine M2	166±31	9.8	95
Clenbuterol	104±5	1.7	104
Enbosarm	105±6	6.1	96
Ligandrol	103±8	7.3	100
Stenabolic	101±11	9.3	111
Stenabolic M2	97±23	6.3 (n=6)	101
Stenabolic M6	105±8	5.5 (n=4)	103
Testolone	89±6	7.0	96
YK-11	133±11	8.1 (n=6)	n.a.

Cardarine had a NESS value of 1.28 µg/L which was 74% lower than the theoretical spiked value. To investigate this, additional experiments were conducted. To assess how filtering and the methanol content affected recovery of cardarine, solutions of 2.5 µg/L were prepared in filtered and acidified wastewater with 10%, 20%, 30%, 50% and 100% MeOH. An additional vial was prepared, where unfiltered wastewater (with 20% MeOH) was fortified to 2.5 µg/L and subsequently filtered. The experiments confirmed that the MeOH content, as well as the filtering process, had an impact on the concentration of cardarine in wastewater. Calculated concentrations for cardarine were as follows: 10% – 1.3 µg/L, 20% (%MeOH in calibration solutions) – 2.4 µg/L, 30% - 4.1 µg/L, 50% - 4.6 µg/L and 100% - 4.6 µg/L, which translates to recoveries of 52%, 96%, 164%, 184%, and 184%, respectively. This suggests that solubility is a contributing factor for this biomarker and results should be interpreted with caution when analysing an aqueous matrix. Another significant amount of cardarine was lost through filtering, as the concentration after filtering (with 20% MeOH) was 0.9 µg/L (vs 2.5 µg/L). This is likely due to its high logP value (5.85) and therefore, having a higher affinity to the particulates and/or filter than the aqueous wastewater. Overall, around 50% was lost through only having 10% MeOH as opposed to the 20% in the calibration, and 63% was lost due to filtration. All other analytes (except glucuronides) were investigated also, and no notable losses were observed with differences in methanol content or the filtering process.

In addition, a subsequent in-sample stability experiment (currently unpublished) revealed that wastewater preservation with HCl led to a 98% lower initial concentration of cardarine than the theoretical spiked value, whereas the initial concentration in unpreserved and sodium metabisulfite preserved wastewater was within the expected range. It is possible that the HCl-preserved wastewater had a pH value slightly below 2.14, which, according to ChemAxon prediction (as reported by DrugBank (<https://go.drugbank.com/drugs/DB05416>, accessed 29/07/2021)), is the strongest basic pKa of cardarine, whereas the pH of the calibrations series solutions may have been slightly above this pH level. As samples were preserved with HCl, this is likely a significant contributing factor, explaining a portion of the 92% decrease in initial concentration for cardarine in this study.

It is difficult to estimate which of these three factors played the largest role in the loss or lower detected concentrations of cardarine, as these are preliminary findings. If this biomarker were to be used in future experiments, losses due to filtering, methanol content in the sample, and pH values should be assessed in more detail.

Initial concentrations in the reactors

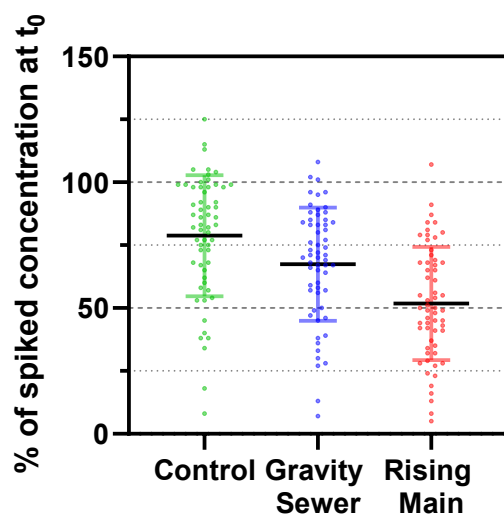


Figure S1. Scatter dot plot of measured concentration of analytes in t_0 as a percentage of the spiked theoretical concentration in all three reactor types with mean (black line) and standard deviation.

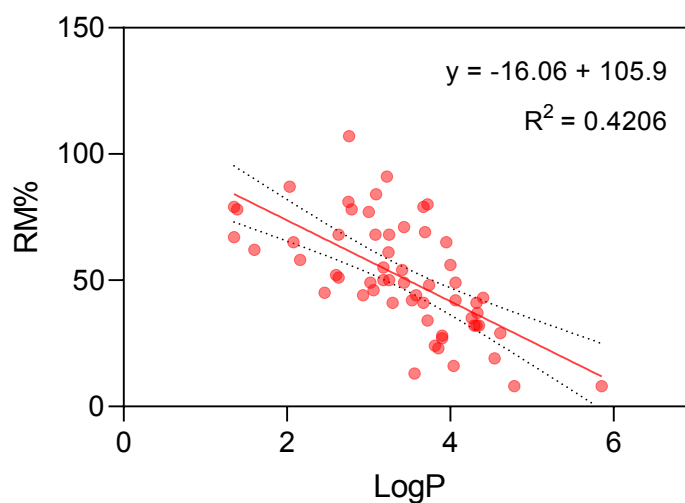


Figure S2. Scatter plot of anabolic agent (N=59) logP value vs their concentration at t_0 divided by the theoretical concentration at which they were spiked (RM%). Red trend line represents a simple linear regression. Black dotted lines represent the 95% confidence interval of the slope.

Stability of metabolites and parent analytes

Table S5. Values for parameters of zero order, first order and two-phase equations for all biomarkers in the control reactor, gravity sewer reactor and rising main reactor. Bolded values are from the model of best fit. For equations and parameter descriptions, please refer to the “Statistical analysis” section.

Analyte	Control reactor								Gravity sewer reactor								Rising main reactor							
	Zero order		First order		Two-phase				Zero order		First order		Two-phase				Zero order		First order		Two-phase			
	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s
Boldenone	-5.165	121.9	121.4	-0.040	-49.2	-1.056	148.6	-0.042	-9.795	100.4	116.1	-0.233	2.7E+07	-0.104	-2.7E+07	-0.104	-6.746	56.9	100.0	-0.906	-5.2E+05	-1.223	5.2E+05	-1.223
Boldenone gluc	-6.633	56.0	100.0	-0.971	4.6E+06	-0.669	-4.6E+06	-0.669	-6.175	51.4	100.0	-1.111	4.4E+05	-0.835	-4.4E+05	-0.835	-4.811	39.1	100.0	-1.999	100.0	-1.998	0.0	-0.158
Boldenone M1	-3.471	92.2	95.8	-0.050	8.4	-2.264	91.7	-0.043	-7.765	86.0	99.5	-0.216	2.2	-67.222	97.8	-0.212	-6.270	53.0	99.9	-1.019	64.3	-1.335	35.8	-0.652
Clostebol M1	-1.396	95.6	96.0	-0.016	5.8	-42.690	94.2	-0.013	-6.480	82.3	92.3	-0.169	27.5	-1.107	72.3	-0.124	-6.294	68.9	89.1	-0.315	54.1	-1.195	45.6	-0.136
Drostanolone M1	-4.402	94.0	96.7	-0.067	15.4	-0.841	86.3	-0.051	-6.716	83.2	95.3	-0.184	60.8	-0.411	38.6	-0.067	-5.635	63.9	88.2	-0.387	71.2	-1.095	28.1	-0.066
Drostanolone M1 gluc	-6.745	97.7	101.5	-0.102	-8.3E+06	-0.162	8.3E+06	-0.162	-9.082	83.1	105.6	-0.340	9.1E+05	-0.181	-9.1E+05	-0.181	-6.307	52.3	100.0	-0.880	9.1E+05	-0.636	-9.1E+05	-0.636
Estrone	3.824	98.7	99.9	-0.007	-4.8	-0.656	103.2	-0.011	-4.727	90.3	95.3	-0.083	45.2	-0.319	56.0	-0.024	-5.009	72.8	82.0	-0.147	52.8	-1.210	46.4	-0.052
Fluoxymesterone	-0.029	98.8	98.9	0.000	98.3	0.002	0.0	2.822	-7.302	97.8	104.6	-0.134	1.1E+07	-0.062	-1.1E+07	-0.062	-7.466	69.3	98.9	-0.489	89.6	-0.594	10.5	-0.131
Fluoxymesterone M1	1.483	96.5	96.8	0.014	98.0	0.008	0.0	2.823	-0.753	98.3	99.0	-0.008	17.3	-0.299	84.4	0.007	-0.496	97.2	97.3	-0.005	7.0	-1.396	93.8	-0.001
Fluoxymesterone M2	-0.721	98.9	98.7	-0.006	98.9	-0.007	0.0	2.829	-4.720	87.7	92.3	-0.088	35.2	-0.472	63.5	-0.039	-5.258	73.1	84.3	-0.170	56.8	-0.959	42.8	-0.051
Mesterolone M1	-5.732	94.5	99.4	-0.104	9.7	-0.624	91.5	-0.089	-6.985	80.9	94.6	-0.212	38.1	-0.633	61.2	-0.138	-6.128	63.0	91.6	-0.487	64.1	-1.287	35.4	-0.147
Methyl-1-testosterone	2.177	116.3	101.6	-0.047	-3.4	-43.398	103.4	-0.049	-7.015	61.1	100.8	-0.542	-5.1E+05	-0.929	5.1E+05	-0.928	-6.411	53.3	100.0	-1.410	18.2	-51.339	81.8	-1.335
Metandienone	1.807	102.5	103.5	0.001	-8.7E+05	-0.060	8.7E+05	-0.060	-8.401	86.6	101.8	-0.254	9.2E+05	-0.156	-9.2E+05	-0.156	-6.495	55.1	99.9	-0.878	99.8	-0.884	0.2	0.014
Metandienone M1	-0.731	101.7	100.4	-0.006	-38.8	-0.099	138.3	-0.024	-8.331	84.3	100.9	-0.278	4.6E+06	-0.200	-4.6E+06	-0.200	-6.016	50.5	100.0	-1.024	15.6	-58.052	84.4	-0.939
Metandienone M2	-0.081	96.7	97.4	-0.001	4.3	-3.310	95.8	0.001	-6.981	96.6	102.4	-0.126	7.4E+05	-0.053	-7.4E+05	-0.053	-7.684	68.3	100.6	-0.568	-5.5	-62.175	105.5	-0.598
Metandienone M3	-2.118	92.9	93.6	-0.027	14.3	-1.923	86.0	-0.017	-7.866	83.0	99.0	-0.252	4.6	-1.581	95.4	-0.241	-6.512	55.9	100.0	-0.858	23.0	-63.181	77.0	-0.598
Methasterone	-1.268	103.9	103.4	-0.013	-4.7E+04	-0.028	4.7E+04	-0.028	-6.033	91.2	97.8	-0.121	90.2	-0.200	10.4	0.073	-5.042	66.9	81.8	-0.220	67.2	-1.475	32.7	-0.034
Methasterone M1	-4.539	91.0	96.3	-0.075	26.0	-0.503	74.4	-0.044	-5.890	84.3	95.0	-0.159	45.3	-0.468	54.6	-0.082	-5.219	60.2	86.0	-0.423	67.5	-1.542	31.9	-0.080
Metenolone	-5.048	99.2	100.4	-0.061	101.8	-0.072	0.0	2.820	-7.869	87.6	98.8	-0.216	96.6	-0.235	3.1	0.013	-6.598	61.8	97.5	-0.637	86.4	-0.922	13.7	-0.109
Metenolone M1	-2.487	96.6	97.4	-0.030	3.8	-52.209	96.2	-0.029	-5.337	86.6	91.9	-0.105	24.0	-0.867	75.3	-0.074	-5.740	71.6	85.5	-0.215	53.2	-1.123	46.5	-0.087

Analyte	Control reactor								Gravity sewer reactor								Rising main reactor							
	Zero order		First order		Two-phase				Zero order		First order		Two-phase				Zero order		First order		Two-phase			
	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s
Methylstenbolone	0.227	103.1	103.2	0.001	-4.5	-9.245	104.5	0.000	-7.018	90.8	99.9	-0.155	96.1	-0.189	5.2	0.051	-6.166	64.1	91.5	-0.456	61.1	-1.687	38.8	-0.149
Methyltestosterone	-2.835	100.6	101.2	-0.033	1.2E+06	-0.004	-1.2E+06	-0.004	-8.568	82.5	102.3	-0.331	7.8E+04	-0.234	-7.8E+04	-0.234	-5.330	43.9	100.0	-1.585	99.0	-1.613	1.0	-0.246
Methyltestosterone M1/Norethandrolone M2	-3.561	95.6	97.2	-0.049	17.1	-0.396	83.2	-0.033	-6.503	85.5	95.2	-0.156	38.1	-0.478	61.5	-0.095	-6.017	65.9	88.2	-0.355	62.2	-1.155	37.2	-0.114
Methyltestosterone M2	-1.698	96.6	96.7	-0.020	12.6	-0.627	88.1	-0.009	-4.467	86.9	91.0	-0.080	37.9	-0.482	60.9	-0.030	-4.985	70.6	80.0	-0.159	49.8	-1.465	49.6	-0.066
Nandrolone	-5.085	100.4	101.1	-0.053	100.7	-0.065	1.3	0.190	-8.569	83.9	103.3	-0.299	1.4E+06	-0.195	-1.4E+06	-0.195	-5.995	50.0	100.0	-1.156	-3.9E+05	-1.293	3.9E+05	-1.293
19-Norandrosterone	-1.503	99.4	99.5	-0.017	99.3	-0.022	0.8	0.184	-5.638	87.8	93.9	-0.113	33.9	-0.473	66.1	-0.066	-5.827	71.0	84.8	-0.223	51.0	-1.265	48.5	-0.101
19-Norandrosterone gluc	-8.414	80.4	100.2	-0.323	3.6E+06	-0.202	-3.6E+06	-0.202	-8.111	71.1	101.4	-0.506	1.5E+07	-0.312	-1.5E+07	-0.312	-5.681	46.6	100.0	-1.274	164.0	-1.104	-64.0	-0.877
19-Noretiocholanolone	-2.466	96.2	97.9	-0.034	12.4	-0.486	88.0	-0.020	-4.427	87.8	92.2	-0.081	40.2	-0.452	59.5	-0.027	-5.182	74.8	84.2	-0.150	50.0	-0.989	49.1	-0.057
19-Noretiocholanolone gluc	-6.243	52.1	100.0	-1.087	8.3E+06	-0.837	-8.3E+06	-0.837	-5.745	47.5	100.0	-1.355	3.4E+05	-1.032	-3.4E+05	-1.032	-4.611	37.3	100.0	-5.194	100.0	-3.216	0.0	0.752
Norethandrolone M1	-1.492	99.3	98.8	-0.012	5.9	-0.595	94.8	-0.008	-6.109	84.8	93.4	-0.142	40.4	-0.474	58.3	-0.077	-5.535	65.1	84.4	-0.303	61.6	-1.344	37.9	-0.090
Norethandrolone M3	-1.020	100.9	100.6	-0.010	100.9	-0.012	0.0	2.816	-3.326	95.5	97.1	-0.045	26.5	-0.309	74.1	-0.017	-4.493	79.9	85.4	-0.098	43.6	-0.853	55.6	-0.035
Progesterone	-8.748	85.3	104.6	-0.307	1.3E+07	-0.203	-1.3E+07	-0.203	-8.169	71.9	100.2	-0.715	-1.7	-47.749	101.7	-0.727	-4.603	37.3	100.0	-5.203	135.8	-2.225	-35.8	-1.891
Oxandrolone	0.582	99.6	98.1	0.007	-2.7E+05	-0.021	2.7E+05	-0.021	-1.857	97.3	98.3	-0.023	60.0	-0.175	44.2	0.045	-3.410	86.8	88.9	-0.054	33.2	-0.763	66.4	-0.017
Oxandrolone M1	0.643	95.0	95.6	0.006	-5.6E+04	-0.016	5.7E+04	-0.016	-1.792	91.2	91.6	-0.023	94.1	-0.036	0.0	0.920	-2.755	79.5	82.4	-0.052	48.8	-0.677	49.1	0.014
Testosterone	-6.694	95.2	99.9	-0.082	100.6	-0.089	0.0	0.484	-8.388	79.1	101.7	-0.363	2.3E+06	-0.256	-2.3E+06	-0.256	-5.295	43.6	100.0	-1.583	8.1	-52.643	91.9	-1.496
Androstenedione	-2.949	79.6	100.4	-0.029	29.1	-2.353	71.0	-0.027	-7.920	73.3	100.6	-0.384	-2.8E+03	-0.544	2.9E+03	-0.536	-4.627	37.6	100.0	-8.219	100.0	-39.279	0.0	-1.881
Androsterone	-4.497	93.0	96.0	-0.069	14.0	-0.652	85.7	-0.054	-6.174	84.2	93.2	-0.149	37.7	-0.570	61.5	-0.086	-5.369	70.9	82.9	-0.191	53.3	-1.217	46.2	-0.074
Androsterone gluc	-7.294	62.7	100.0	-0.678	110.6	-0.635	-10.8	-0.340	-6.874	57.7	100.3	-0.804	8.7E+05	-0.544	-8.7E+05	-0.544	-5.011	40.7	100.0	-1.718	8.9E+05	-1.117	-8.9E+05	-1.117
Epitestosterone	-2.180	96.9	97.2	-0.023	4.3	-4.289	95.7	-0.022	-8.307	80.0	100.9	-0.330	4.6E+05	-0.243	-4.6E+05	-0.243	-4.935	40.4	100.0	-1.913	96.2	-1.800	3.8	-0.719
Etiocholanolone	-2.181	89.9	91.0	-0.030	43.2	-0.417	56.7	0.031	-1.575	92.4	93.0	-0.020	16.2	-0.783	83.1	-0.006	-1.437	99.2	99.4	-0.016	100.2	-0.020	0.0	2.817
Stanozolol M1	-0.831	95.6	95.9	-0.011	23.3	-0.251	76.6	0.012	-5.641	88.5	95.2	-0.114	39.4	-0.459	61.5	-0.056	-5.221	67.0	81.8	-0.228	62.3	-1.192	37.0	-0.058
Stanozolol M1 gluc	-4.822	39.2	100.0	-2.055	100.4	-2.043	-0.4	-0.711	-4.429	35.8	100.0	-2.474	100.0	-40.807	0.0	-2.295	-4.154	33.4	100.0	-6.053	100.0	-35.703	0.0	-2.858
Trenbolone	-2.432	103.6	100.0	-0.009	103.6	-0.042	0.0	1.139	-7.178	90.5	99.4	-0.170	70.5	-0.422	30.8	-0.032	-6.002	67.8	92.6	-0.364	78.6	-0.925	21.2	-0.028

Analyte	Control reactor								Gravity sewer reactor								Rising main reactor							
	Zero order		First order		Two-phase				Zero order		First order		Two-phase				Zero order		First order		Two-phase			
	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s	k ₀	e	f _f	k _f	f _f	k _f	f _s	k _s
Trenbolone M1	-0.426	96.5	96.6	-0.005	5.0	-4.457	95.0	-0.003	-4.363	89.8	93.8	-0.074	31.4	-0.449	68.0	-0.034	-4.509	81.6	86.3	-0.093	37.0	-0.988	62.5	-0.045
DHCMT	-0.729	102.6	102.0	-0.006	-15.4	-0.275	115.4	-0.019	-8.529	91.0	99.8	-0.254	116.9	-0.198	-17.6	-0.089	-6.976	60.4	100.0	-0.802	10.4	-54.245	89.6	-0.723
DHCMT M1	-0.417	97.6	97.6	-0.004	3.9	-47.800	96.1	-0.002	-7.160	96.3	102.9	-0.133	4.1E+06	-0.069	-4.1E+06	-0.069	-8.116	71.5	101.3	-0.490	5.1E+07	-0.307	-5.1E+07	-0.307
Andarine	-1.473	99.9	100.1	-0.016	-6.6E+06	-0.076	6.6E+06	-0.076	-8.470	81.1	102.6	-0.339	1.2E+06	-0.228	-1.2E+06	-0.228	-5.922	50.1	100.0	-1.010	18.2	-54.042	81.8	-1.084
Cardarine	1.156	96.7	93.8	0.018	96.6	-0.052	4.4	0.250	-5.747	94.9	93.5	-0.096	68.7	-0.264	33.9	0.004	-6.115	90.2	85.0	-0.113	50.9	-0.851	49.0	-0.029
Cardarine M1	-1.183	92.3	93.2	-0.016	12.0	-2.753	88.2	-0.009	-3.026	88.8	90.4	-0.044	16.3	-2.473	83.7	-0.033	-4.549	87.3	91.6	-0.084	88.8	-0.179	8.6	0.120
Cardarine M2	-0.169	92.2	92.5	-0.003	21.6	-1.233	79.2	0.020	-4.232	95.9	98.9	-0.066	68.6	-0.148	32.8	0.012	-7.106	85.7	99.1	-0.193	92.9	-0.264	8.7	0.035
Clenbuterol	-0.115	103.0	100.1	-0.003	-3.7	-11.050	103.7	-0.003	-2.532	95.4	96.3	-0.032	25.7	-0.325	74.4	-0.005	-4.077	81.3	85.4	-0.082	42.9	-0.826	56.9	-0.024
Enbosarm	-1.235	97.0	97.8	-0.015	3.7	-2.064	96.3	-0.013	-5.251	85.5	91.9	-0.107	46.0	-0.378	52.5	-0.041	-5.105	68.3	82.1	-0.195	63.4	-1.153	35.4	-0.043
Ligandrol	-1.057	96.6	97.3	-0.012	6.4	-1.560	94.3	-0.007	-4.906	86.5	92.3	-0.095	55.3	-0.318	44.4	-0.016	-4.835	69.1	79.4	-0.160	59.8	-1.059	39.4	-0.041
Stenabolic	-6.212	81.5	93.7	-0.163	13.9	-5.666	86.1	-0.144	-8.156	77.5	100.6	-0.381	100.9	-0.383	0.0	0.423	-5.686	48.0	100.0	-1.173	78.3	-1.584	21.7	-0.455
Stenabolic M2	-7.877	104.8	108.8	-0.124	2.7E+06	-0.004	-2.7E+06	-0.004	-7.349	63.3	99.9	-0.672	1.8E+04	-0.496	-1.8E+04	-0.495	-4.031	32.4	100.0	-9.179	100.0	-6.118	0.0	2.702
Stenabolic M6	-9.275	93.0	108.2	-0.249	5.1E+05	-0.114	-5.1E+05	-0.114	-5.710	47.3	100.0	-1.265	24.8	-52.821	75.2	-1.090	-3.710	29.6	100.0	-10.560	1.2E+04	-3.283	-1.2E+04	-3.281
Testolone	-0.624	101.0	100.4	-0.006	56.1	-0.016	44.5	0.005	-5.207	91.0	97.4	-0.098	76.0	-0.235	27.3	0.023	-5.206	72.2	85.7	-0.179	66.9	-0.842	33.7	-0.025
YK-11	-1.927	96.1	98.5	-0.024	-5.2E+06	-0.057	5.2E+06	-0.057	-7.876	81.1	98.7	-0.258	97.9	-0.251	0.0	2.776	-5.907	49.4	100.0	-1.110	6.6E+05	-0.837	-6.6E+05	-0.837
Paracetamol	-0.112	99.8	100.0	-0.002	0.4	-0.563	99.6	-0.002	-6.865	98.8	104.0	-0.118	3.1E+07	-0.046	-3.1E+07	-0.046	-7.492	65.5	100.3	-0.586	-1.9E+06	-0.889	1.9E+06	-0.889
Acesulfame	0.081	100.6	100.1	0.001	-0.5	-2.618	100.5	0.001	-0.089	101.6	100.8	0.000	-1.3	-43.282	101.3	-0.001	-0.028	99.8	99.9	0.000	100.3	-0.002	0.0	2.821

Steroid glucuronides

Table S6. Initial (t_0) concentrations of glucuronides, maximum measured concentrations of deconjugated biomarker, and maximum deconjugation of glucuronides (%) in CR, GS, and RM. The percent of initial glucuronide concentration that was transformed was determined at the maximum value observed for its non-glucuronidated form over 12h.

Glucuronide	DRO M1*	19-NA*	19-NE*	ADS*	STZ M1*
t_0 concentration [CR] ($\mu\text{g/L}$)	5.8	9.8	9	12.9	3.3
t_0 concentration [GS] ($\mu\text{g/L}$)	5.2	8.4	7	10	2.2
t_0 concentration [RM] ($\mu\text{g/L}$)	5.2	7.9	6.7	9.4	2.2
Maximum measured concentration of non-glucuronide [CR] ($\mu\text{g/L}$)	0.9	4.7	5.6	2.1	2.9
Maximum measured concentration of non-glucuronide [GS] ($\mu\text{g/L}$)	0.6	2.9	4.3	1.9	2.2
Maximum measured concentration of non-glucuronide [RM] ($\mu\text{g/L}$)	0.4	1.9	3.1	1.6	1.4
t_0 concentration transformed [CR]	16%	48%	62%	16%	88%
t_0 concentration transformed [GS]	12%	35%	61%	19%	100%
t_0 concentration transformed [RM]	8%	24%	46%	17%	64%

*DRO M1: Drostanolone M1, 19-NA: 19-Norandrosterone, 19-NE: 19-Noretiocholanolone, ADS: Androsterone, STZ M1: Stanozolol M1

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