Supplementary Material

A Screening Method for the Quantitative Determination of Selective Androgen Receptor Modulators (SARMs) in Capsules by High Resolution ^{19}F- and ^{1}H-NMR Spectroscopy

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p3 Figure S1. Mass spectrometry: extracted ion chromatograms (EIC) of the SARMs reference standards.

p4 Figure S2. Superimposition of the total ion chromatograms of the SARMs samples (full line) with the reference standards EIC (dotted line). The good overlapping of the full line and the dotted line, and the absence of signals in the blank chromatogram (not shown), confirmed the selectivity of the MS method.

p5 Figure S3. Comparison of the experimental isotope distribution of the SARMs (Panel A) with the corresponding theoretical distribution (Panel B). The good match of the m/z distributions supported identification.

p6 Figure S4. ^{1}H, ^{13}C, ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC spectra of Cardarine reported as an example. **Panel A**: Zoom of the ^{1}H spectrum. The signals of the five non-equivalent proton groups are present in the 8.7-6.5 ppm aromatic range. The singlet at about 9.7 ppm was assigned to the carboxyl proton; **Panel B**: Zoom of the ^{13}C spectrum. The numerous signals detected in the 140-120 ppm range are partly due to the splitting caused by carbon-fluorine coupling; **Panel C**: Zoom of the ^{13}C spectrum evidencing the signal quartets of the carbons coupled to fluorines. The quartets are evidenced by blue brackets. The signal of the carbon directly bonded to fluorine is centred at about 124 ppm and shows a 272.4 Hz carbon-fluorine coupling (CF_3, $J_{C\text{-}F}$=272.4 Hz). The quartet at 129 ppm belongs to the carbon one bond apart from fluorine (C4", $J_{C\text{-}F}$=34.0 Hz) and the tight quartet at about 126 ppm was assigned to the carbons two bonds apart from fluorine (C3",C5" $J_{C\text{-}F}$=3.7 Hz); **Panel D:** Superimposed ^{1}H-^{13}C HMBC (blue) and ^{1}H-^{13}C HSQC (green/pink) spectra. The pink and green cross peaks represent proton correlation with CH_2 and CH/CH_3 groups respectively. The blue cross peaks are representative of the structural carbon backbone. It is noteworthy that each of two methyl groups found at about 2 ppm selectively correlates to the closer aromatic ring in the structure (circled in red).

p8 Figure S5. Comparison between SARMs extraction performed in EtOH (red) vs DMSO (blue). Results are reported as % in respect to the declared content.

p9 Figure S6. Overlapped 1H- NMR spectra obtained by the pulse programs: zg (blue), zgpr (red), noesygppr1d (green), cpmgpr1d (purple). The SARMs spectra are coded as follows: Andarine (panel A), Cardarine (panel B), Ligandrol (panel C), Ostarine (panel D), S-23 (panel E).

p10 Figure S7. qNMR linearity for ^{19}F-zgig30 (blue profile), ^{1}H-zg (orange profile) and ^{1}H-zgpreq (green profile) per SARM. The linear regression equation and R^2 are reported for each NMR experiment.

p11 Figure S8. Trend of the residuals of qNMR linearity for ^{19}F-zgig30 (blue profile), ^{1}H-zg (orange profile) and ^{1}H-zgpreq (green profile) per SARM. All residuals are within ±2σ as evidenced in the inserts.

p12 Table S1. Acquisition and processing parameters of ^{19}F- and ^{1}H-qNMR analytical methods

p13 Table S2. Summary of Mass Spectrometry results.

p14 Table S3. Repeatability obtained by six consecutive measurements of each SARM by ^{19}F-NMR. Results are expressed as mg/capsule. RSD threshold limit was 1.5%.

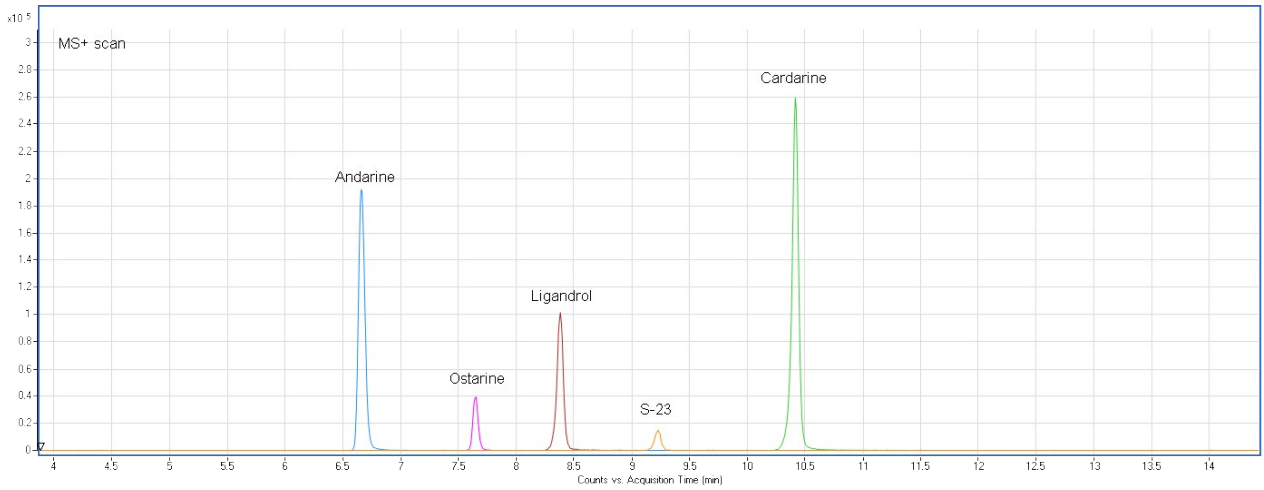
p15 Table S4. Intermediate precision of ^{19}F-qNMR. Each sample content is reported as mg/cps. The results were obtained from integrations of the same samples performed by three different operators. The RSD is within 3.0% for all the experiments.

p16 Table S5. Precision (RSD) of the results obtained on three samples for each NMR experiment. The % is referred to the intermediate weight. All the weights are within the linearity range.

p17 Table S6. Linear and Multiple Regression and Correlation of the pulse programs ^{19}F-zgig30, ^{1}H-zg, ^{1}H-zgpreq.

p18 Table S7. LOD and LOQ per SARM referred to each pulse program.

p19 Table S8. Robustness: acquisition parameters and results for the following pulse programs: ^{19}F-zgig30 (upper section), ^{1}H-zg (intermediate section) and ^{1}H-zgpreq (lower section).



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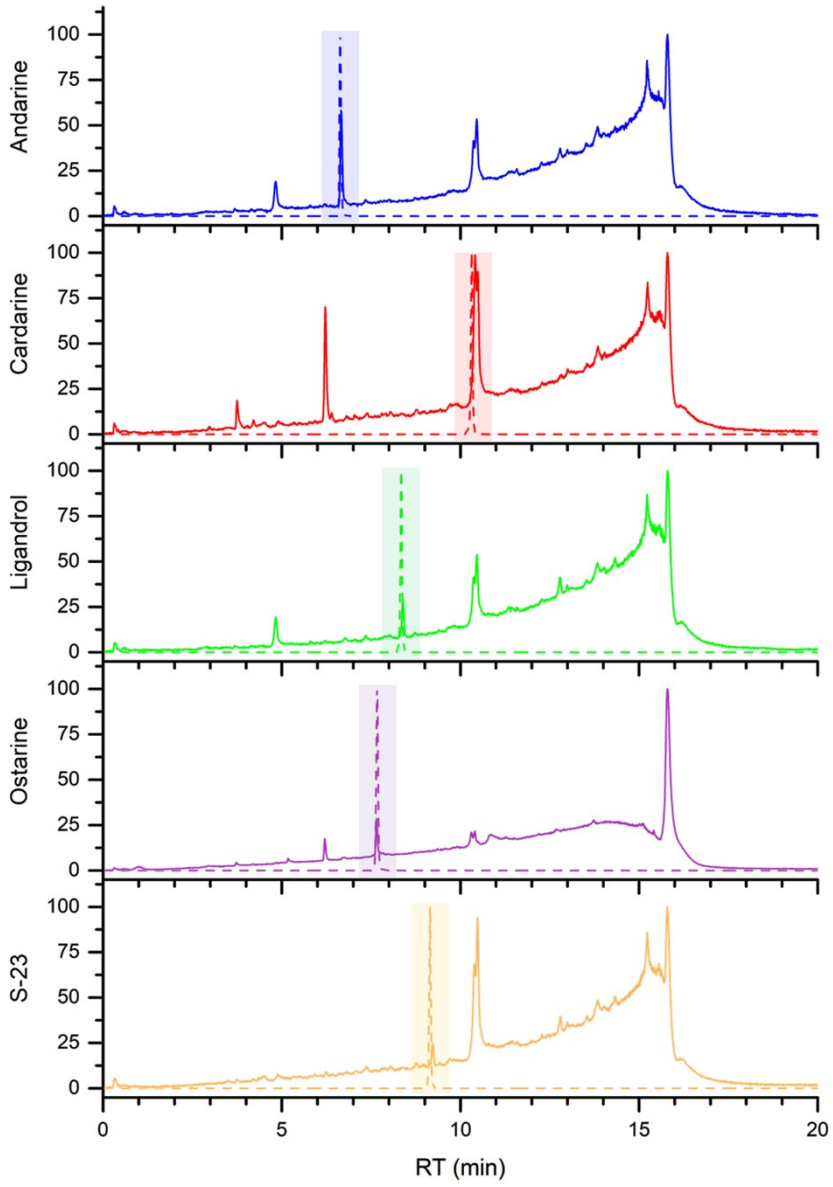

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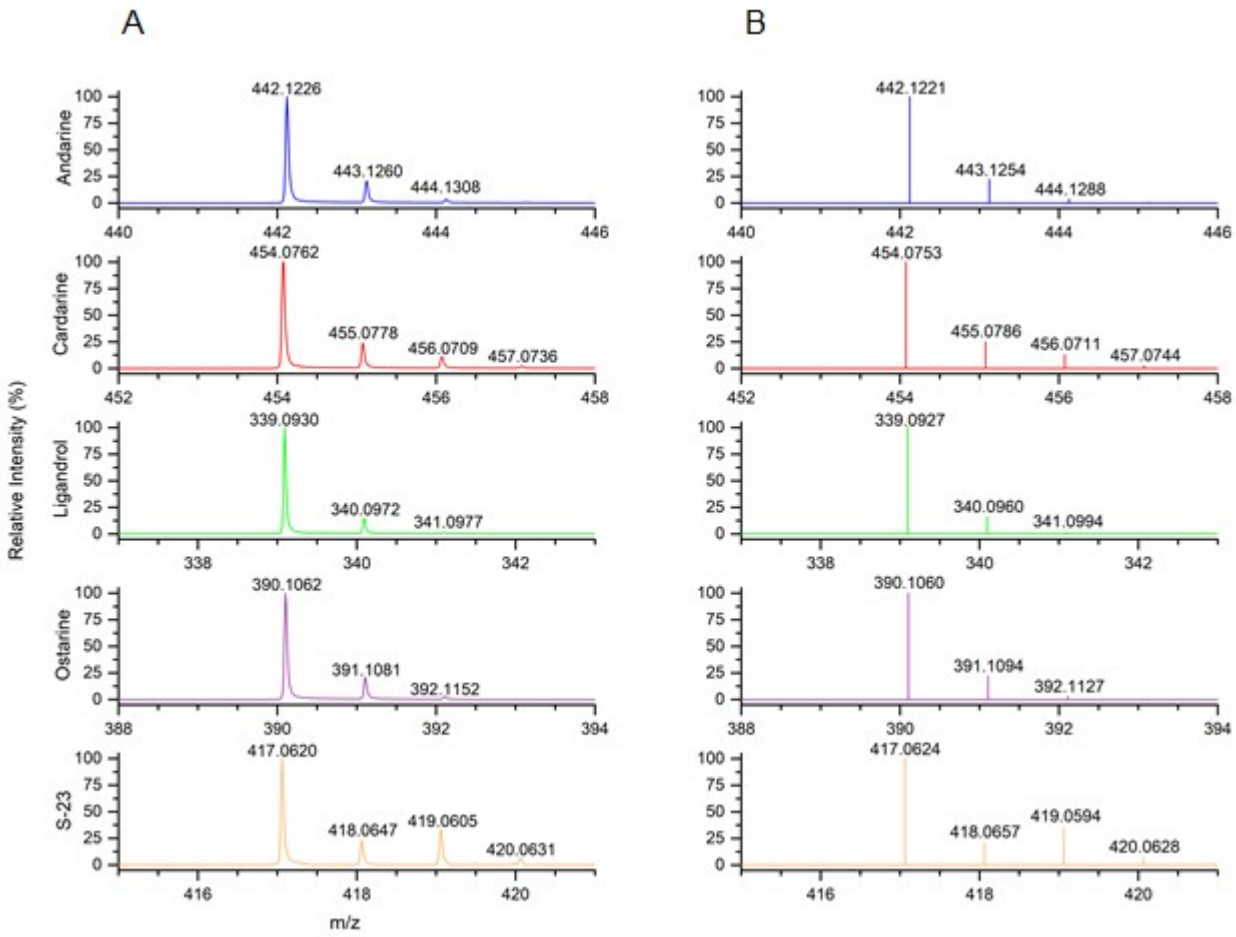

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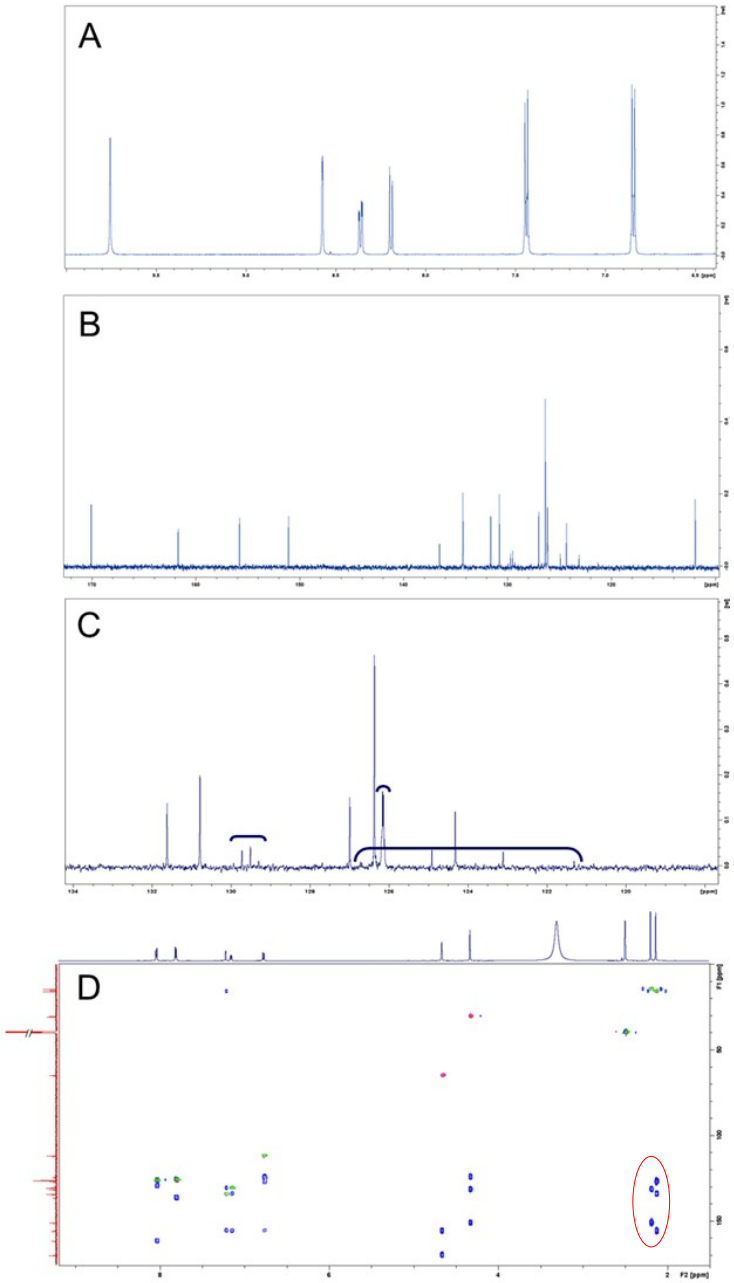

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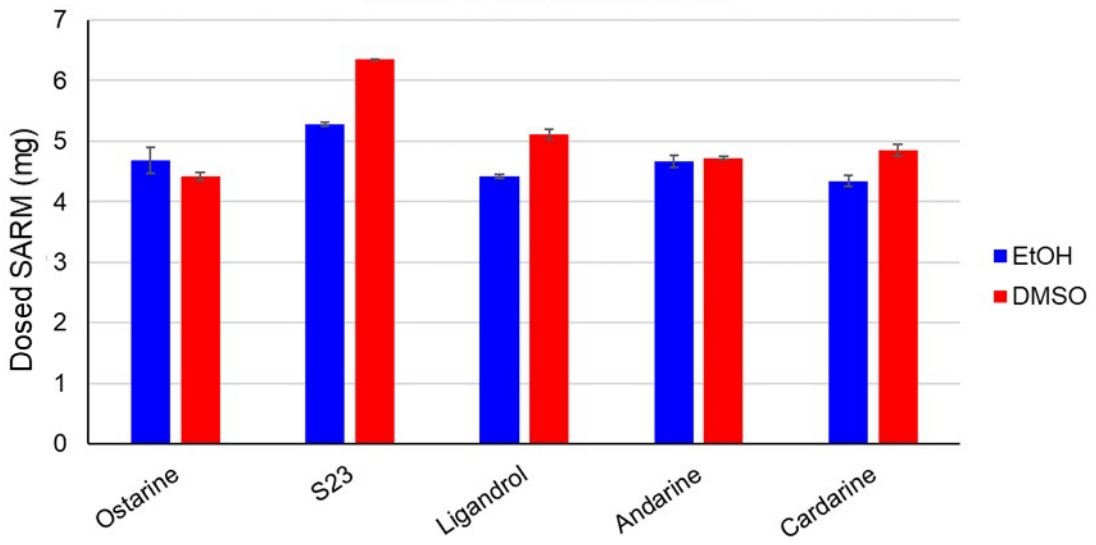

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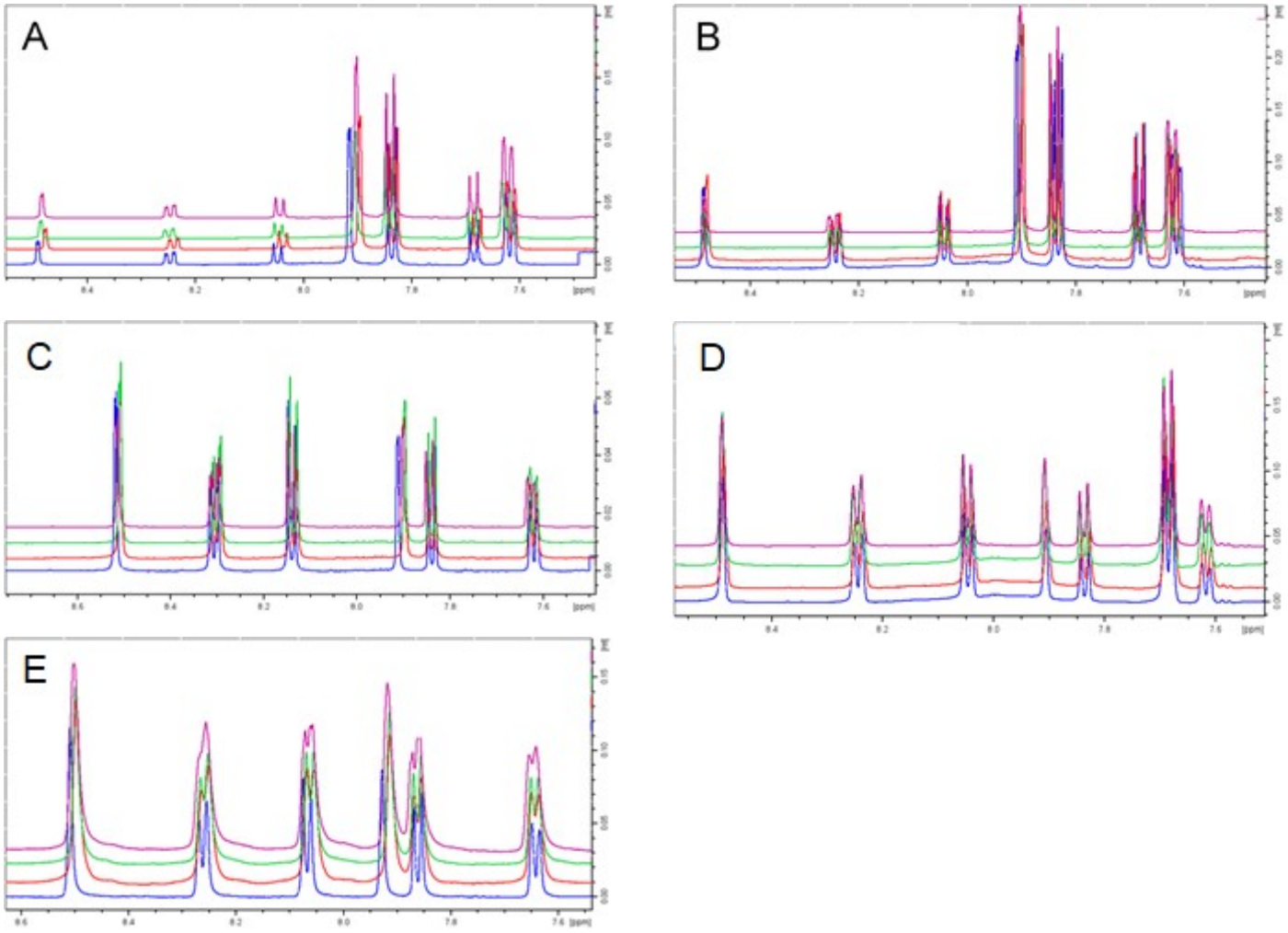

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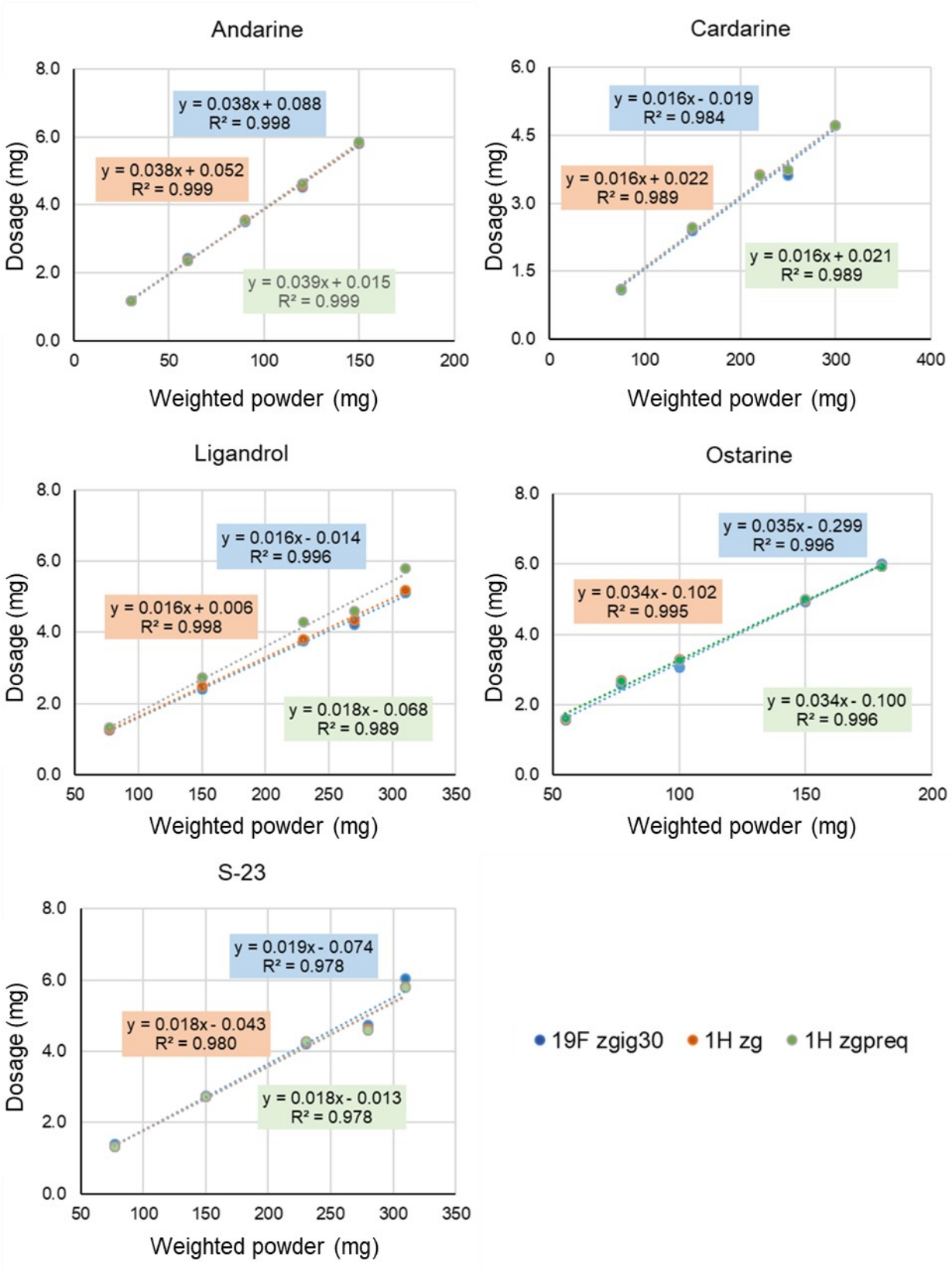

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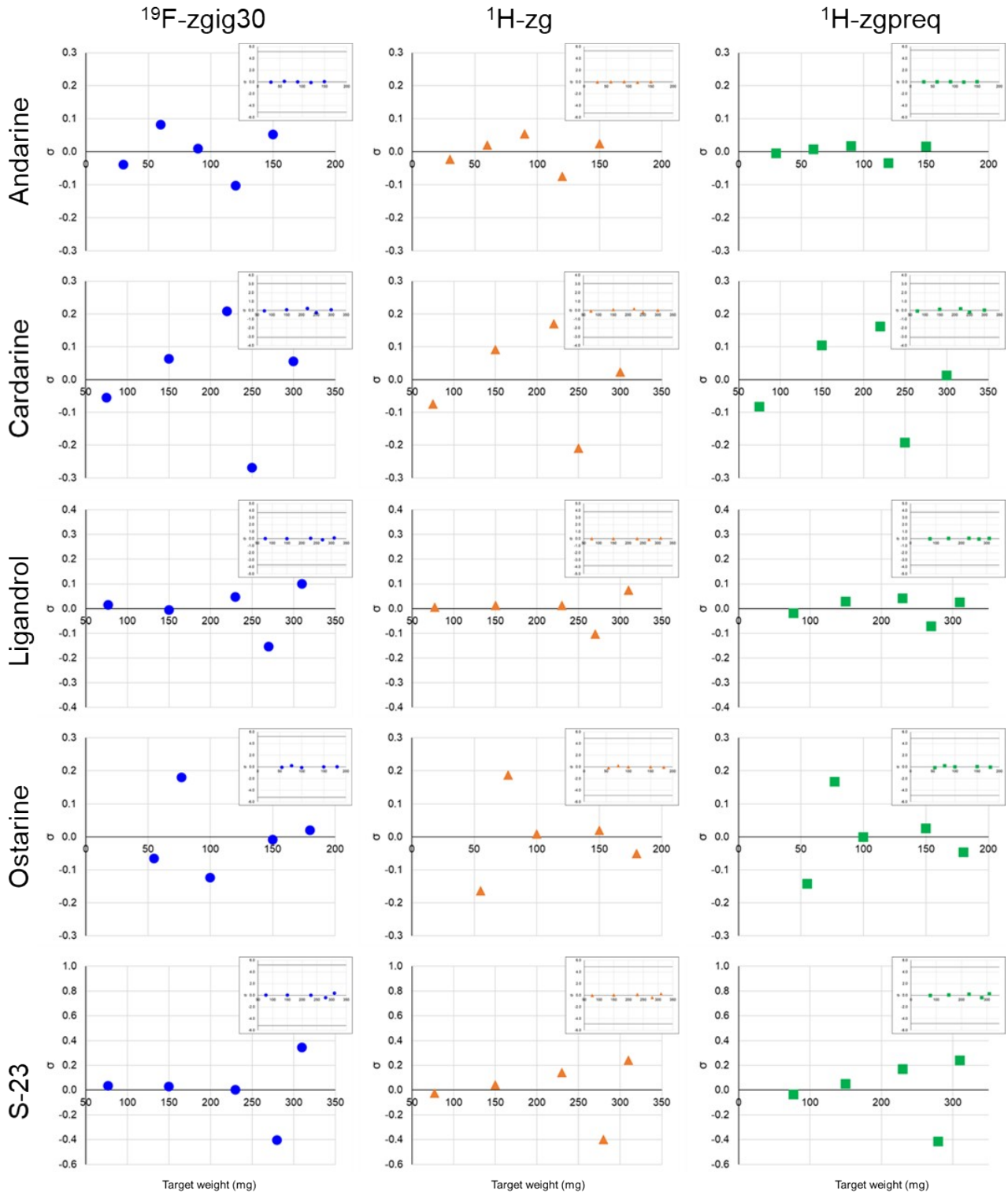

Figure S8. Trend of the residuals of qNMR linearity for ^{19}F zgig30 (blue profile), ^{1}H zg (orange profile) and ^{1}H zgpreq (green profile) per SARM. All residuals are within ±2σ

Table S1. Acquisition and processing parameters of ^{19}F- and ^{1}H-qNMR analytical methods

^{19}F-zgig30			
Pulse angle	30°	NS	24
Pulse strength	15 dB	Size of FID	128 k
Pulse length	4 µs	Size of real spectrum	512 k
Measurement Temperature	298 K	Line broadening	0.3 Hz
Pre-acquisition delay	6.50 µs	Expt	3.75 min
Acquisition time	1.2 s		
Relaxation delay d1	10 s		
O1P	-65.0 ppm		
^{1}H-zg			
Pulse angle	90°	NS	24
Pulse strength	12 dB	Size of FID	64 k
Pulse length	12 µs	Size of real spectrum	512 k
Measurement Temperature	298 K	Line broadening	0.3 Hz
Pre-acquisition delay	6.50 µs	Expt	19.1 min
Acquisition time	2.8 s		
Relaxation delay (d1)	45 s		
O1P	2.5 ppm		
^{1}H-zgpreq			
Pulse angle	90°	NS	24
Pulse strength	12 dB	Size of FID	64 k
Pulse length	12 µs	Size of real spectrum	512 k
Measurement Temperature	298 K	Line broadening	0.3 Hz
Preacquisition delay	6.50 µs	PLW[9]	6.8e-05 W
Acquisition time	2.8 s	Expt	19.5 min
Relaxation delay (d9)	45 s		
Solvent suppression delay (d1)	1 s		
O1P	-65.0 ppm		

Table S2. Summary of Mass Spectrometry results

AI	MF[a]	m/z_{exp}[b]	m/z_{theo}[b]	Δppm	RT_{Sample}	RT_{STD}	MS/MS[c]	
							m/z_{exp}[b]	Rel. Intens. (%)
Andarine	$C_{19}H_{18}F_3N_3O_6$	442.1226	442.1221	1.1	6.66	6.64	208.097	100.0
							190.086	42.1
							108.045	29.0
							166.086	18.8
Cardarine	$C_{21}H_{18}F_3NO_3S_2$	454.0762	454.0753	2.0	10.46	10.33	454.075	100.0
							257.048	47.0
Ligandrol	$C_{14}H_{12}F_6N_2O$	339.0930	339.0927	0.9	8.37	8.34	339.092	100.0
							199.048	16.8
Ostarine	$C_{19}H_{14}F_3N_3O_3$	390.1062	390.1060	0.5	7.66	7.67	370.099	100
							120.044	33.9
							251.061	29.1
							271.069	14.5
							193.021	17.3
							187.046	10.9
S-23	$C_{18}H_{13}ClF_4N_2O_3$	417.0620	417.0624	-0.9	9.23	9.16	187.045	100.0
							185.016	38.9
							203.026	15.5
							213.023	14.6
							188.050	7.4

a MF stands for Molecular Formula. All SARMs were detected as protonated species.
b m/z_{exp} and m/z_{theo} stand for experimental and theoretical m/z ratio, respectively.
c MS/MS experiments were performed with a collision energy = 20V

Table S3. Repeatability obtained by six consecutive measurements of each SARM by ^{19}F-NMR. Results are expressed as mg/capsule. The RSD acceptance criteria was 1.5%.

Dosed SARM (mg/cps)	#1	#2	#3	#4	#5	#6	Mean	Std Dev	RSD
Andarine	19.2	19.1	19.0	19.2	19.1	19.2	19.1	0.05	0.28
Cardarine	7.5	7.5	7.5	7.6	7.6	7.6	7.5	0.05	0.70
Ligandrol	8.8	8.7	8.9	8.8	8.9	8.8	8.8	0.06	0.68
Ostarine	13.1	13.2	13.1	13.2	13.0	13.1	13.1	0.04	0.29
S-23	9.4	9.4	9.2	9.3	9.4	9.3	9.3	0.06	0.61

Table S4. Intermediate precision of ^{19}F-qNMR. Each sample content is reported as mg/cps. The results were obtained from integrations of the same samples performed by three different operators. The RSD is within 3.0% for all the experiments.

	Operator 1	**SD**	**Operator 2**	**SD**	**Operator 3**	**SD**	**SD total**	**Mean**	**RSD%**
Andarine	20.1	0.33	19.7	0.3	20.2	0.1	0.2	20.0	1.0
Cardarine	7.8	0.03	7.8	0.1	8.1	0.2	0.1	8.0	1.7
Ligandrol	8.6	0.18	8.3	0.3	8.9	0.2	0.2	8.6	2.7
Ostarine	13.5	0.06	13.7	0.2	13.5	0.2	0.1	13.6	0.7
S-23	9.3	0.10	9.1	0.1	9.2	0.1	0.1	9.2	0.8

Table S5. Precision (RSD) of the results obtained on three samples for each NMR experiment. The % is referred to the intermediate weight. All the weights are within the linearity range.

SARM	Sample Weight	RSD		
		^{19}F-zgig30	**^{1}H-zg**	**^{1}H-zgpreq**
Andarine	67%, 100%, 167%	0.5	1.4	1.6
Cardarine	50%, 100%, 167%	2.9	3.8	0.9
Ligandrol	65%, 100%, 135%	1.6	0.5	2.2
Ostarine	77%, 100%, 180%	1.5	0.4	0.4
S-23	51%, 100%, 206%	2.7	3.5	3.2

Table S6. Linear and Multiple Regression and Correlation of the pulse programs ^{19}F-zgig30, ^{1}H-zg, ^{1}H-zgpreq.

Pulse program	Linearity regression	SARMs				
		Andarine	**Cardarine**	**Ligandrol**	**Ostarine**	**S-23**
^{19}F-zgig30	R^2	0.998	0.989	0.996	0.996	0.978
	Slope	0.038	0.016	0.016	0.035	0.019
	Intercept	0.088	0.021	-0.014	-0.299	-0.074
	Standard Error	0.085	0.160	0.110	0.132	0.307
	Residual sum square	0.022	0.077	0.036	0.052	0.283
	F	1780.253	296.287	765.699	745.505	134.497
^{1}H-zg	R^2	0.999	0.989	0.998	0.995	0.981
	Slope	0.038	0.0156	0.016	0.034	0.0181
	Intercept	0.052	0.0221	0.006	-0.102	-0.043
	Standard Error	0.058	0.169	0.074	0.147	0.282
	Residual sum square	0.010	0.0859	0.017	0.065	0.239
	F	3938.362	264.121	1735.157	563.260	150.290
^{1}H-zgpreq	R^2	0.999	0.989	0.999	0.996	0.978
	Slope	0.039	0.016	0.016	0.034	0.018
	Intercept	0.015	0.022	0.018	-0.100	-0.013
	Standard Error	0.025	0.169	0.054	0.130	0.295
	Residual sum square	0.002	0.086	0.009	0.0510	0.262
	F	21878.430	264.121	3277.896	717.001	135.010
Multiple Regression on combined results from three pulse programs	R^m	0.999	0.994	0.999	0.998	0.999
	Standard Error	0,053	0.178	0.077	0.128	0.290
	F	4633,85	237.97	1589.66	757.87	924.99
	F significance	0.000	0.000	0.000	0.000	0.001
Correlation	^{19}F-zgig30 - ^{1}H-zg	0.999	0.999	0.999	0.999	0.999
	^{19}F-zgig30 - ^{1}H-zgpreq	0.999	0.999	0.999	0.999	0.999
	^{1}H-zg - ^{1}H-zgpreq	0.999	1	0.999	0.999	0.999

Table S6. LOD and LOQ per SARM referred to each pulse program.

		Andarine	Cardarine	Ligandrol	Ostarine	S-23
Pulse program	**C (mg/ml)**	1.69	1.70	1.69	2.67	2.06
	NS	16	16	16	16	16
	RG	101	101	101	101	101
^{19}F-zgig30	**S/N**	481	345	191	140	365
	LoD (mg/ml)	0.01	0.01	0.03	0.06	0.02
	LoQ (mg/ml)	0.03	0.05	0.09	0.19	0.06
	NS	24	24	24	24	24
	RG	1.94	1.67	1.94	1.74	1.94
^{1}H-zg	**S/N**	453	69	91	140	197
	LoD (mg/ml)	0.01	0.07	0.05	0.06	0.03
	LoQ (mg/ml)	0.04	0.24	0.18	0.19	0.10
	NS	24	24	24	24	24
	RG	17.37	11.54	22.35	10.3	8.92
^{1}H-zgpreq	**S/N**	599	135	219	172	340
	LoD (mg/ml)	0.01	0.04	0.02	0.05	0.02
	LoQ (mg/ml)	0.03	0.12	0.08	0.15	0.06

TableS7. Robustness: acquisition parameters and results for pulse programs: ^{19}F-zgig30 (upper section), ^{1}H-zg (intermediate section) and ^{1}H-zgpreq (lower section). Recovery is reported as % in respect to the results obtained by the reference experiment (reported in bold).

		^{19}F-zgig30			
		Ostarine		**Ligandrol**	
Parameter		**Dosed (mg/cps)**	**Recovery (%)**	**Dosed (mg/cps)**	**Recovery (%)**
O1P	-50 ppm	13.6	99.8	8.6	96.8
	-65 ppm	**13.6**	**100.0**	**8.8**	**100.0**
	-80 ppm	13.7	100.2	8.9	100.8
p1	11 µs	13.6	99.8	9.0	101.8
	12 µs	**13.6**	**100.0**	**8.8**	**100.0**
	13 µs	13.6	99.5	8.5	96.3
PCPD2	60 µs	13.7	100.5	8.5	96.3
	70 µs	**13.6**	**100.0**	**8.8**	**100.0**
	80 µs	13.6	100.0	8.7	97.9

		^{1}H-zg			
		Ostarine		**Ligandrol**	
Parameter		**Dosed (mg)**	**Recovery (%)**	**Dosed (mg)**	**Recovery (%)**
O1P	2.4 ppm	13.6	100.0	9.0	101.9
	2.5 ppm	**13.6**	**100.0**	**8.8**	**100.0**
	2.6 ppm	13.6	99.9	9.1	102.8
p1	9 µs	13.6	100.0	9.1	102.6
	10 µs	**13.6**	**100.0**	**8.8**	**100.0**
	11 µs	13.5	99.3	9.3	104.6

		^{1}H-zgpreq			
		Ostarine		**Ligandrol**	
Parameter		**Dosed (mg)**	**Recovery (%)**	**Dosed (mg)**	**Recovery (%)**
d1	0.5 s	13.8	101.5	9.1	103.3
	1 s	**13.6**	**100.0**	**8.8**	**100.0**
	1.5 s	13.6	99.5	9.1	103.0
p1	9 µs	13.5	99.3	9.0	102.0
	10 µs	**13.6**	**100.0**	**8.8**	**100.0**
	11 µs	13.5	99.2	9.0	101.3