Supplementary Materials: Quantitative Metabolomic Analysis of Urinary Citrulline and Calcitroic acid in Mice after Exposure to Various Types of Ionizing Radiation

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Table S1: Experimental LC-MS conditions for citrulline and calcitroic acid

UPLC column	ACQUITY UPLC BEH HILIC. 1.7µm, 2.1mm × 100mm
Injection volume (µL)	5
Column temperature (°C)	45
Autosampler injection mode	Partial loop needle overfill (PLNO)

Table S2: Tune page parameters

Instrument	Xevo-TQS
Capillary voltage (kV)	3
Cone voltage (V)	30
Source temperature (°C)	150
Desolvation temperature (°C)	500
Cone gas flow (L/Hr)	150
Desolvation gas (L/Hr)	900
Sample temperature (°C)	4
Mode of ionization	Positive

Table S3: Mobile phase gradient for citrulline. Solvent A is acetonitrile with 0.2% formic acid and solvent B is water with 0.2% formic acid.

Step	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.4	99	1	Initial
2	1	0.4	2	98	6
3	3	0.4	2	98	6
4	3.2	0.4	99	1	6
5	5	0.4	99	1	6

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2. Please list all the Figures and Tables in order such as Figure S1, Figure S2…..
3. Please provide captions of all the Figures.

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Table S4: Mobile phase gradient for calcitroic acid. Solvent A is methanol with 0.1% formic acid and solvent B is water with 0.2% formic acid.

Step	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.4	80	20	Initial
2	2	0.4	50	50	6
3	2.5	0.4	50	50	6
4	2.9	0.4	80	20	6
5	5	0.4	80	20	6

Table S5: Citrulline experimental linear range

Sr. No	Sample Text	Type	Std. Conc (ng/mL)	RT	Area	Conc. (ng/mL)	% Deviation*
1	Standards_1	Standard	1	1.34	4267.336	1	4.8
2	Standards_10	Standard	10	1.34	26768.11	9.3	-6.8
3	Standards_100	Standard	100	1.34	274634.8	100	0
4	Standards_250	Standard	250	1.34	710569.1	255.4	2.2
5	Standards_500	Standard	500	1.34	1356185	503.2	0.6
6	Standards_1000	Standard	1000	1.34	2666926	992.1	-0.8

*%Deviation is the difference in experimentally measured standard solution concentration and the nominal concentration expressed in percentage.

Table S6: Calcitroic acid experimental linear range

Sr. No	Sample Text	Type	Std. Conc (ng/mL)	RT	Area	Conc. (ng/mL)	% Deviation*
1	Standards_100	Standard	100	0.74	6371.396	101.5	1.5
2	Standards_250	Standard	250	0.74	16147.66	259.5	3.8
3	Standards_500	Standard	500	0.73	29251.68	471.1	-5.8
4	Standards_750	Standard	750	0.73	43637.5	703.5	-6.2
5	Standards_1000	Standard	1000	0.74	66250.73	1068.8	6.9
6	Standards_2000	Standard	2000	0.74	123612.5	1995.5	-0.2

*%Deviation is the difference in experimentally measured standard solution concentration with respect to that of nominal concentration expressed in percentage.

Table S7: Citrulline recovery study using QC samples

Sample Text	Std. Conc (ng/mL)	RT	Area	IS Area	Response	Height	IS Height	Height/Area	S/N	Conc. (ng/mL)	%Deviation*
QC-1	7.5	1.34	28329.67	120122.1	0.236	427982	1824037	15.107	367.705	9.2	22.5
QC-2	75	1.34	245405.9	127474.5	1.925	3690705	1938244	15.039	2339.951	79.2	5.6
QC-2	75	1.34	252426.9	135619.4	1.861	3812417	2058032	15.103	1643.134	76.2	2
QC-2	75	1.34	262149.8	139131.1	1.884	3947007	2118620	15.056	2047.953	77.5	3.3
QC-2	75	1.34	269430.7	143847.8	1.873	4055174	2189952	15.051	3303.92	77	2.7
QC-3	750	1.34	1947188	111118.2	17.524	30251604	1705529	15.536	2381.648	725.4	-3.3

*%Deviation is the difference in experimentally measured standard solution concentration and the nominal concentration expressed in percentage.

Table S8: Calcitroic acid recovery study using QC samples

Sample Text	Type	Std. Conc (ng/mL)	RT (min)	Area	Response	Height	Conc. (ng/mL)	% Deviation*
QC-1	QC	120	0.75	6845.377	6845.377	61058	109.2	-9
QC-2	QC	1200	0.74	78885.273	78885.273	655633	1272.9	6.1
QC-2	QC	1200	0.74	78180.914	78180.914	645773	1261.6	5.1
QC-2	QC	1200	0.74	72488.844	72488.844	594802	1169.6	-2.5
QC-2	QC	1200	0.74	72617.211	72617.211	594443	1171.7	-2.4
QC-3	QC	1800	0.73	105384.79	105384.789	866361	1701	-5.5

*% Deviation is the difference in experimentally measured standard solution concentration and the nominal concentration expressed in percentage.

Table S9: Citrulline accuracy results

Sr. No	Conc. Level*	Conc (ng/mL)	RT (min)	Area	IS Area	Response	Conc. (ng/mL)	% Deviation**	Average
1		1	1.08	5824.181	657.379	8.86	0.80	-20.0	
2		1	1.08	5806.071	643.988	9.016	1.10	10.0	
3	LLOQ	1	1.07	3111.904	346.241	8.988	1.10	10.0	-2.98
4		1	1.08	5246.309	584.452	8.976	1.00	0.0	
5		1	1.08	3582.166	402.461	8.901	0.90	-10.0	
6		3	1.07	4342.951	444.201	9.777	3.00	0.0	
7		3	1.08	4606.813	468.814	9.827	3.10	3.3	
8	Low QC	3	1.07	5005.619	518.435	9.655	2.70	-10.0	0.58
9		3	1.07	4259.939	433.799	9.82	3.10	3.3	
10		3	1.07	3947.511	396.971	9.944	3.40	13.3	
11		400	1.07	184528.1	944.781	195.313	446.40	11.6	
12		400	1.07	177638.5	962.83	184.496	420.50	5.1	
13	Middle QC	400	1.07	192496	995.858	193.297	441.60	10.4	7.3
14		400	1.07	213086.3	1095.628	194.488	444.40	11.1	
15		400	1.07	197411.7	1140.784	173.049	393.20	-1.7	
16		800	1.07	354614.7	978.262	362.495	846.00	5.7	
17		800	1.07	336069.1	969.694	346.572	807.90	1	
18	High QC	800	1.07	313254.4	910.373	344.095	802.00	0.3	2.42
19		800	1.07	329387.8	877.368	375.427	876.90	9.6	
20		800	1.07	304175	926.697	328.236	764.10	-4.5	

*LLOQ: Lower limit of quantification (1ng/mL); low QC: Lower quality control (3 ng/mL); medium QC: Medium quality control (400 ng/mL); high QC: High quality control (800 ng/mL).

**% Deviation is the difference in experimentally measured standard solution concentration and the nominal concentration expression in percentage.

Table S10: Within-run precision study for citrulline

Sr. No	Conc. Level*	Conc. (ng/mL)	RT (min)	Area	IS Area	Response	Conc. (ng/mL)	Standard Deviation	Mean	% CV
1		1	1.08	5824.181	657.379	8.86	0.8			
2		1	1.08	5806.071	643.988	9.016	1.1			
3	LLOQ	1	1.07	3111.904	346.241	8.988	1.1	0.12	0.98	11.9
4		1	1.08	5246.309	584.452	8.976	1			
5		1	1.08	3582.166	402.461	8.901	0.9			
6		3	1.07	4342.951	444.201	9.777	3			
7		3	1.08	4606.813	468.814	9.827	3.1			
8	Low QC	3	1.07	5005.619	518.435	9.655	2.7	0.22	3.06	7.3
9		3	1.07	4259.939	433.799	9.82	3.1			
10		3	1.07	3947.511	396.971	9.944	3.4			
11		400	1.07	184528.1	944.781	195.313	446.4			
12		400	1.07	177638.5	962.83	184.496	420.5			
13	Middle QC	400	1.07	192496	995.858	193.297	441.6	20.26	429.22	4.7
14		400	1.07	213086.3	1095.628	194.488	444.4			
15		400	1.07	197411.7	1140.784	173.049	393.2			
16		800	1.07	354614.7	978.262	362.495	846			
17		800	1.07	336069.1	969.694	346.572	807.9			
18	High QC	800	1.07	313254.4	910.373	344.095	802	38.75	819.38	4.7
19		800	1.07	329387.8	877.368	375.427	876.9			
20		800	1.07	304175	926.697	328.236	764.1			

*LLOQ: Lower limit of quantification (1ng/mL); low QC: Lower quality control (3 ng/mL); medium QC: Medium quality control (400 ng/mL); high QC: High quality control (800 ng/mL).

Table S11: Inter-batch repeatability

Sr. No	Conc. Level*	Conc. (ng/mL)	Run-1 Conc.	Run-2 Conc.	Run-3 Conc.	Average conc. (ng/mL)	Standard Deviation	Mean	% CV
1		1	0.9	0.9	0.9	0.9			
2		1	1	0.9	1	1			
3	LLOQ	1	0.9	1	1.1	1	0.03	1	3.6
4		1	0.9	0.9	1.1	1			
5		1	0.9	1	0.9	0.9			
6		3	2.9	3.4	3	3.1			
7		3	3	3.3	3.1	3.1			
8	Low QC	3	2.8	3.3	2.7	2.9	0.1	3.1	3.4
9		3	3.4	2.9	3.4	3.2			
10		3	3	3.3	2.7	3			
11		400	458	447.6	433.5	446.4			
12		400	448.8	419.5	453.3	440.5			
13	Middle QC	400	439.7	439.9	411.8	430.5	12.38	430.3	2.9
14		400	447.3	417.1	403.2	422.5			
15		400	455	401.5	378.8	411.8			
16		800	881.2	737.4	712.5	777			
17		800	877.1	827.4	710.8	805.1			
18	High QC	800	881.8	756.8	799.9	812.8	28.29	815.8	3.5
19		800	889.9	746.8	822.2	819.6			
20		800	893.1	825.4	874.7	864.4			

*LLOQ: Lower limit of quantification (1ng/mL); low QC: Lower quality control (3 ng/mL); medium QC: Medium quality control (400 ng/mL); high QC: High quality control (800 ng/mL).

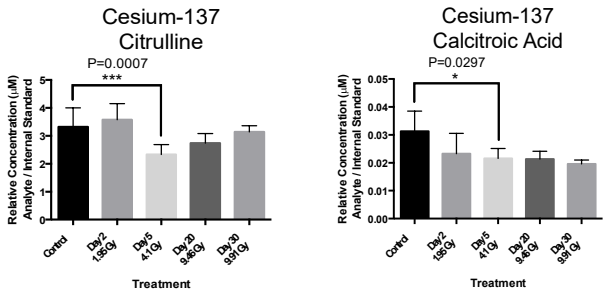

Figure S1. Decrease in urinary excretion of citrulline and calcitoic acid after internal exposure to Cesium-137 at different time-points in a 30-day study. (***) denotes statistical significance of the change in urinary excretion 0f citrulline in terms of *p*-value (*P*=0.0007) while (*) denotes that of calcitroic acid (*P*=0.0297) 5 days after the exposure.

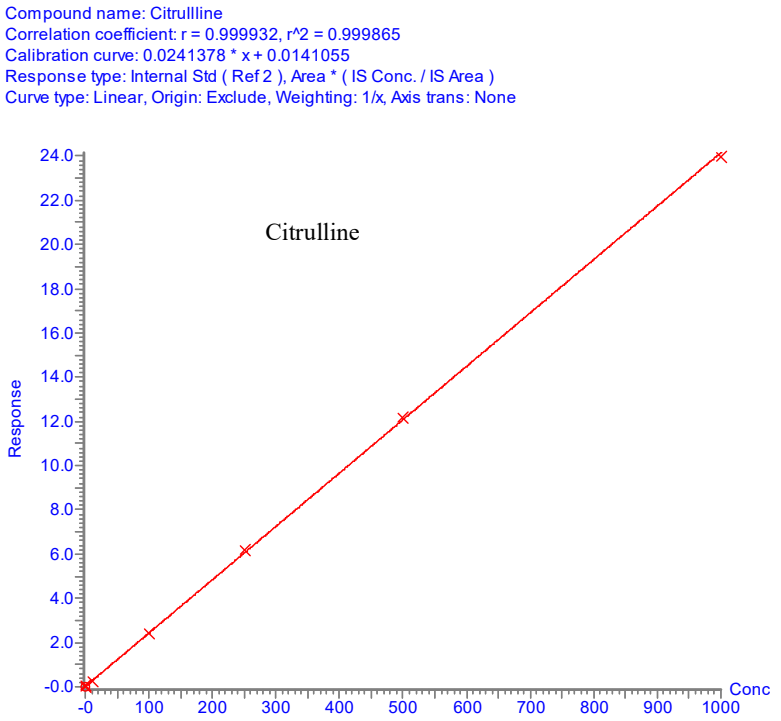

Figure S2. The linear concentration range of the calibration curve for citrulline ranging from ng/mL to 1000 ng/mL).

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Compound name: Calcitroic acid
Correlation coefficient: r = 0.998206, r^2 = 0.996415
Calibration curve: 61.9024 * x + 86.7689
Response type: External Std, Area
Curve type: Linear, Origin: Exclude, Weighting: 1/x, Axis trans: None

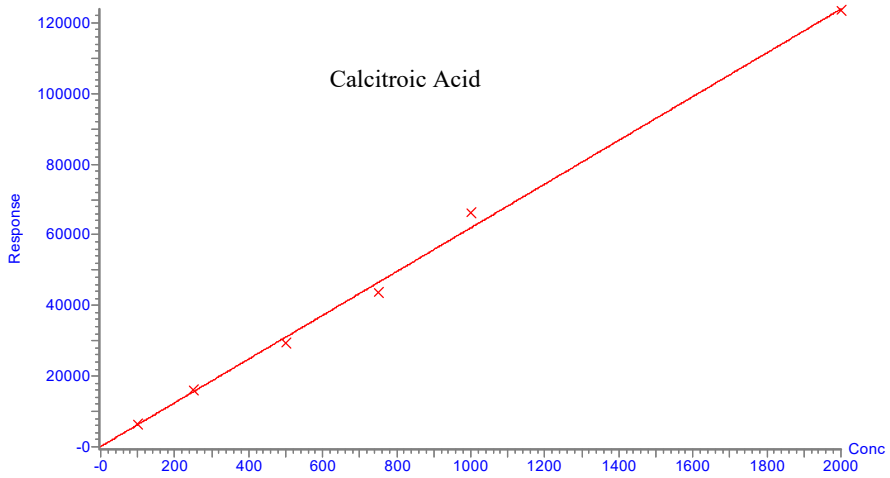

Figure S3. The linear concentration range of the calibration curve for calcitroic acid ranging from 100ng/mL to 2000 ng/mL).

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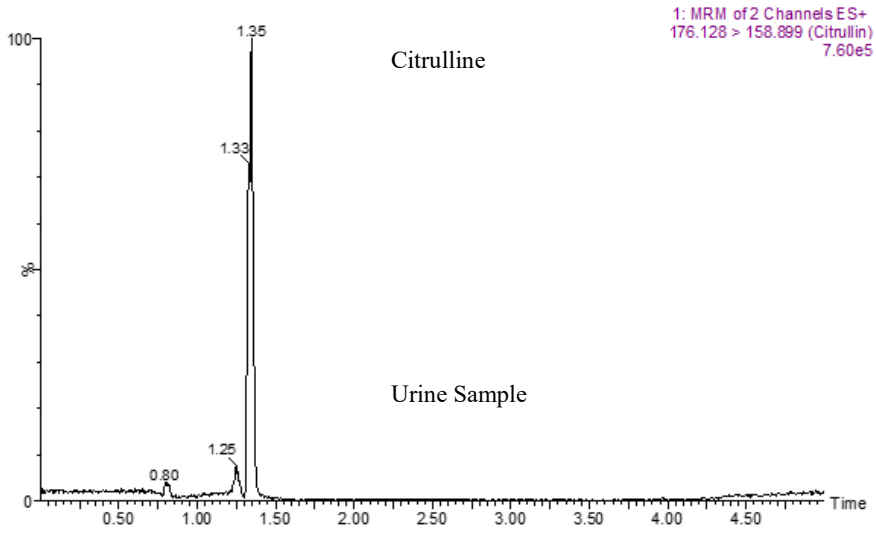
A



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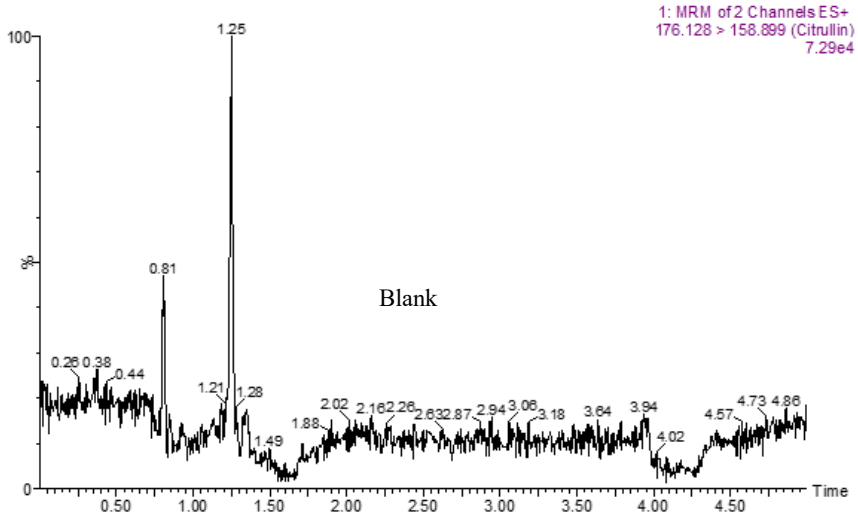

Figure S4. Test (A) and blank (B) chromatograms for citrulline.

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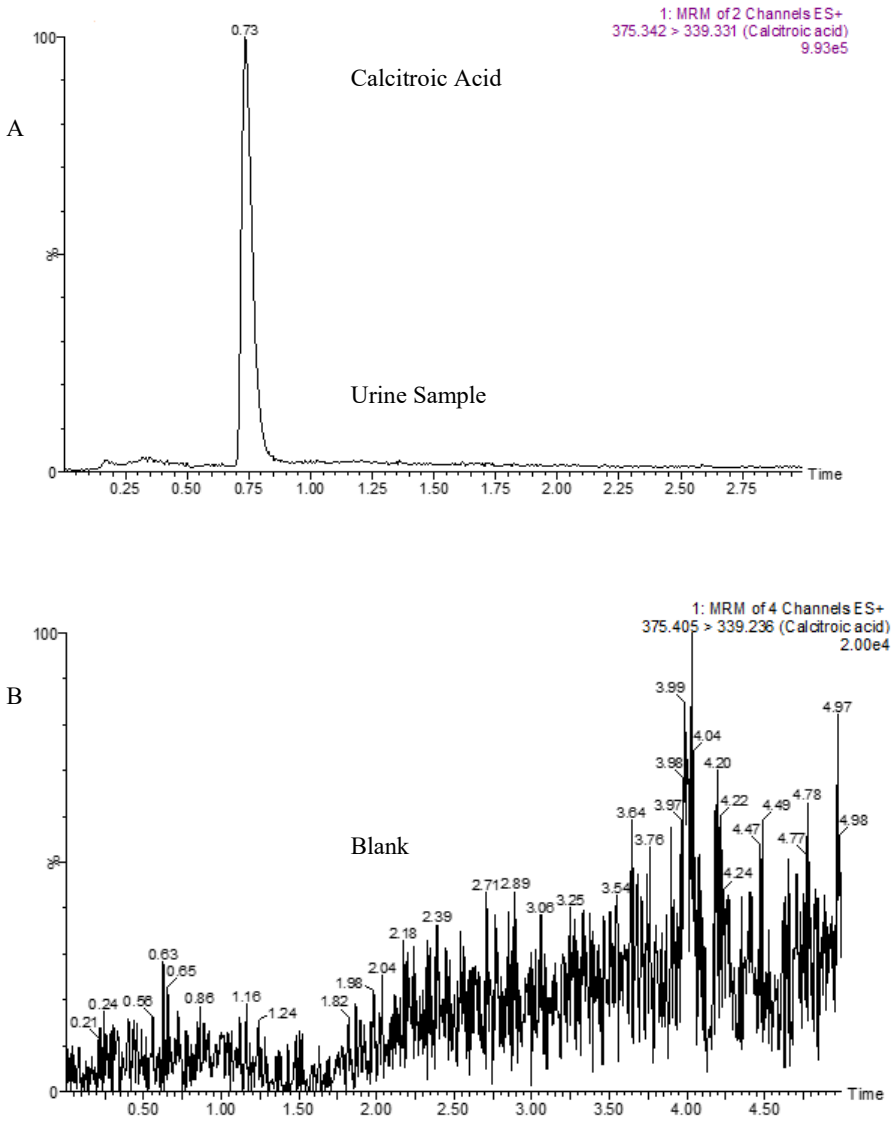

Figure S5. Test (A) and blank (B) chromatograms for calcitroic acid.

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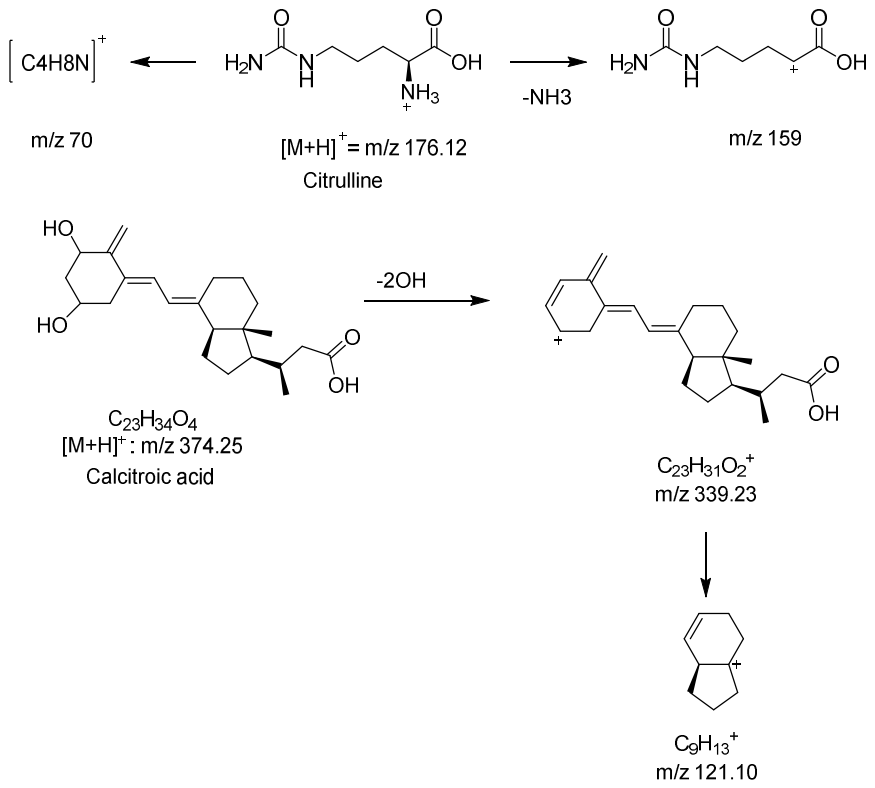

Figure S6. Fragment structures for citrulline and calcitroic acid transitions.