Electronic Supplementary Material (ESI) for Analyst.


# A Quantitative Metabolomics Peek into Planarian Regeneration

**Nivedita Natarajan, Padma Ramakrishnan, Vairavan Lakshmanan[1] , Dasaradhi Palakodeti[1], Kannan Rangiah***

*Metabolomics Facility, Centre for Cellular and Molecular Platforms, [1]Institute of Stem Cell Biology and Regenarative Medicine, National Centre for Biological Sciences, GKVK, Bellary Road, Bangalore-560065, India.*

* Corresponding author: Metabolomics facility, Centre for Cellular and Molecular Platforms, GKVK, Bellary Road, Bangalore-560065, India.

Fax: + 91-80-2363 6662, E-mail:kannanr@ccamp.res.in

# Supplementary Tables and Figures

## Table S1: Stability of derivatives.

| Amines | Stability (Hour) | | |
|---|---|---|---|
| | 0 | 24 | 48 |
| Hydroxy proline | 96.924 | 99.074 | 104.002 |
| Histidine | 100.469 | 101.977 | 97.555 |
| Asparagine | 101.850 | 96.277 | 101.872 |
| Taurine | 96.262 | 99.557 | 104.181 |
| Serine | 99.359 | 100.704 | 99.938 |
| Arginine | 97.083 | 94.580 | 108.337 |
| Homoserine | 98.233 | 98.451 | 103.316 |
| Histamine | 94.010 | 99.600 | 106.390 |
| Aspartic acid | 100.116 | 98.610 | 101.274 |
| Sarcosine | 102.243 | 99.327 | 98.430 |
| Citrulline | 99.896 | 97.541 | 102.564 |
| Glutamic acid | 99.190 | 99.621 | 101.189 |
| Threonine | 97.076 | 98.976 | 103.948 |
| Alanine | 102.086 | 100.519 | 97.395 |
| Aminoadipic acid | 99.637 | 100.046 | 100.317 |
| GABA | 99.293 | 99.835 | 100.872 |
| Proline | 96.786 | 99.907 | 103.308 |
| Epinephrine | 105.418 | 93.622 | 100.960 |
| Norepinephrine | 99.947 | 100.261 | 99.793 |
| DOPA | 100.047 | 99.901 | 100.052 |
| Octapamine | 97.493 | 107.271 | 95.236 |
| Ornithine | 102.212 | 107.192 | 90.596 |
| Lysine | 96.470 | 98.015 | 105.515 |
| Tyrosine | 95.604 | 103.733 | 100.663 |
| Methionine | 96.604 | 98.668 | 104.728 |
| Dopamine | 102.674 | 101.506 | 95.820 |
| Valine | 97.135 | 99.597 | 103.269 |
| Serotonin | 98.621 | 101.420 | 99.959 |
| Tyramine | 94.602 | 99.615 | 105.783 |
| Melotonin | 101.472 | 98.164 | 100.364 |
| Isoleucine | 97.269 | 98.387 | 104.344 |
| Leucine | 97.209 | 99.387 | 103.404 |
| Phenylalanine | 101.513 | 99.947 | 98.540 |
| Tryptophan | 98.936 | 98.244 | 102.820 |
| Tryptamine | 97.288 | 97.548 | 105.164 |
| Cystine | 101.250 | 96.580 | 102.170 |
| Cysteine | 106.754 | 91.355 | 101.892 |
| Cystathionine | 99.958 | 99.362 | 100.679 |
| GSH | 107.075 | 101.672 | 91.253 |
| GSSG | 100.148 | 101.215 | 98.637 |
| Cys-gly | 107.214 | 98.276 | 94.511 |
| γ-glu-cys | 101.113 | 100.077 | 98.809 |

**Table S2: Recovery of metabolites from extraction cartridges.**

| Amino acids | Recovery (%) |
|---|---|
| **Histidine** | 104.506 |
| **Serine** | 90.668 |
| **Arginine** | 75.865 |
| **Glutamic acid** | 94.473 |
| **Threonine** | 89.176 |
| **Proline** | 80.411 |
| **Lysine** | 100.734 |
| **Tyrosine** | 93.422 |
| **Valine** | 74.090 |
| **Methionine** | 92.283 |
| **Luecine** | 83.259 |
| **Isoleucine** | 83.259 |
| **Phenylalanine** | 90.605 |
| **Proline** | 80.411 |

**Figure S1**

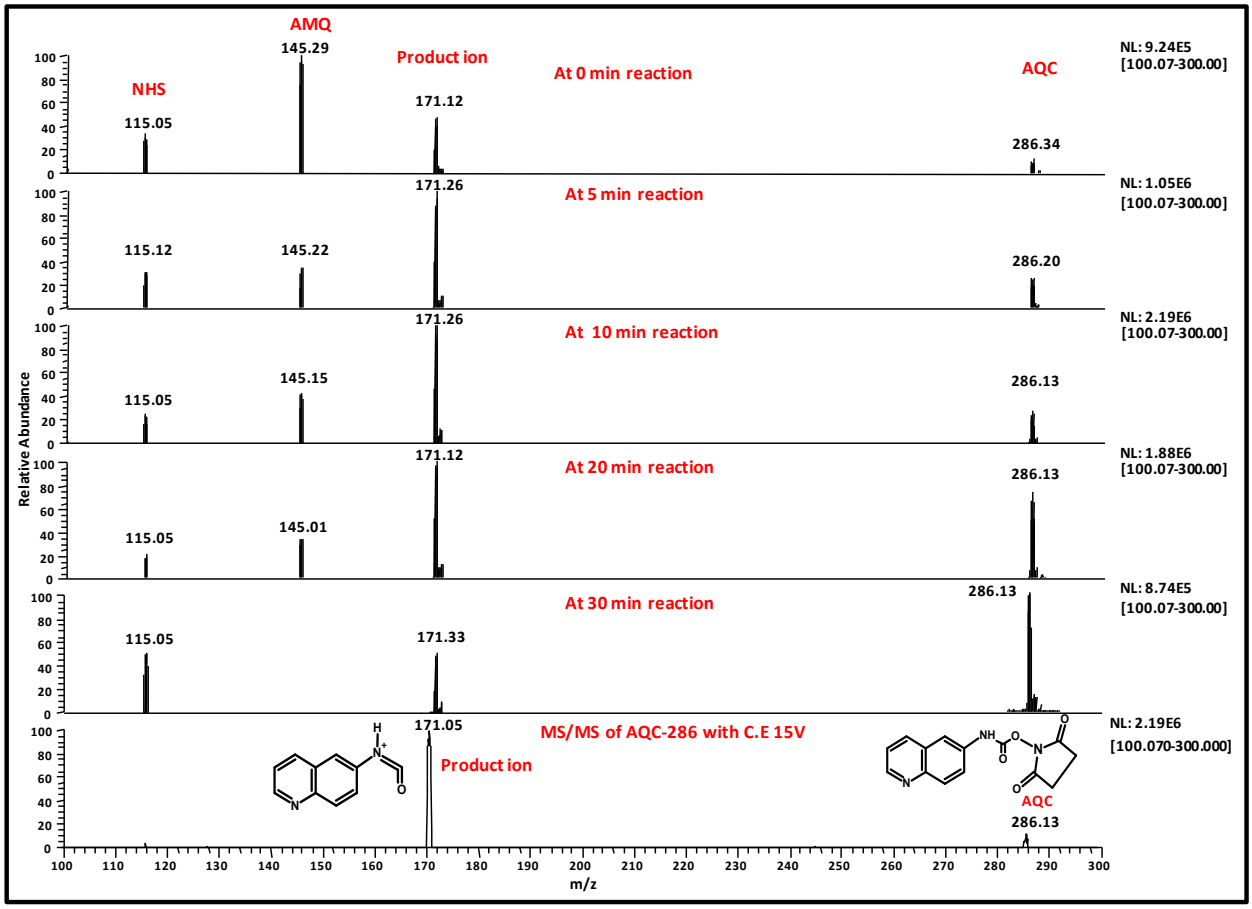



**Figure S1: Synthesis of AQC derivative.** The reaction of 6-Aminoquinoline (AMQ-145 m/z) and N,N′-Disuccinimidyl carbonate (DSC-257 m/z) at 60 °C for 30 min leads to the formation of 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC-286 m/z). N-hydroxysuccinamide (NHS-115 m/z) is the byproduct in the reaction. The MS/MS analysis of AQC by applying by applying collision energy leads to the formation of intense product ion mainly from AQC part.

**Figure S2**

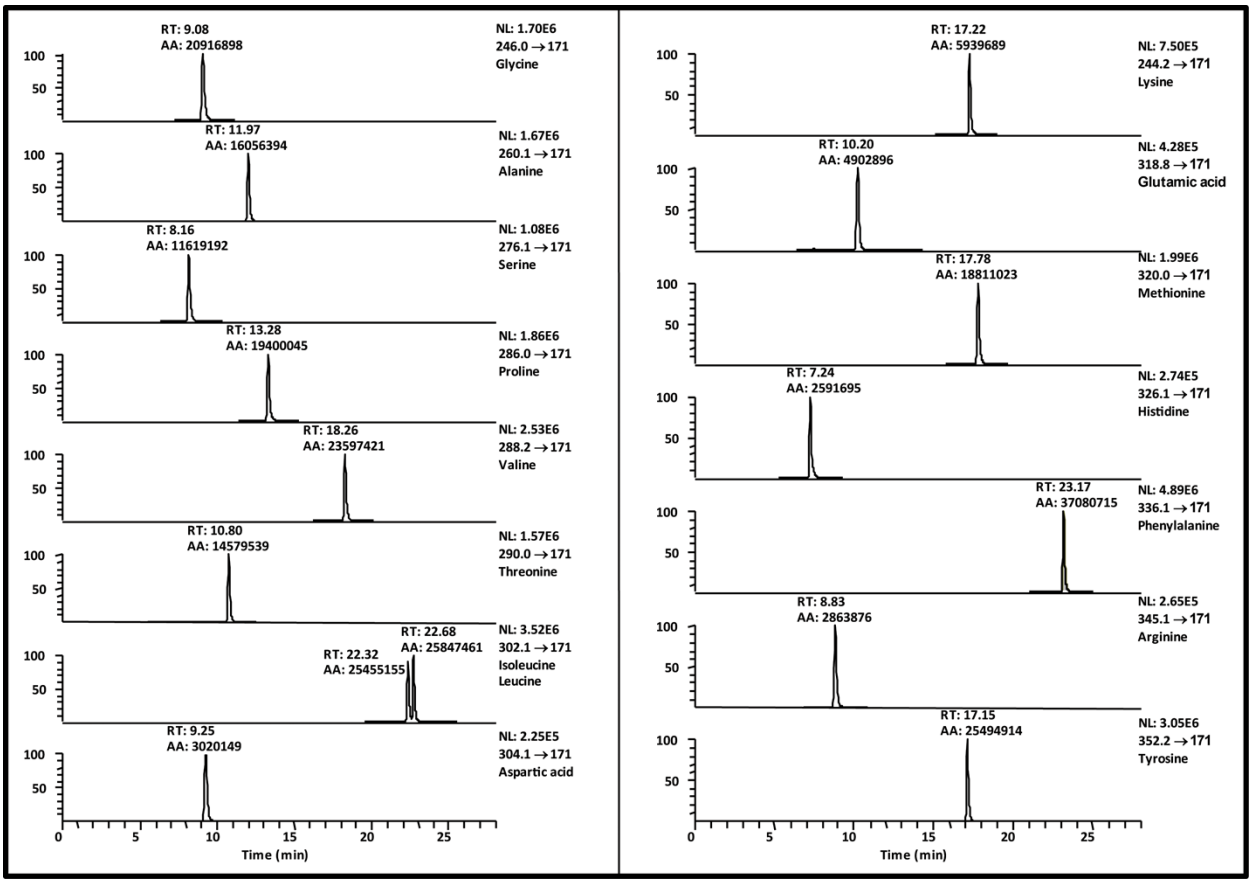
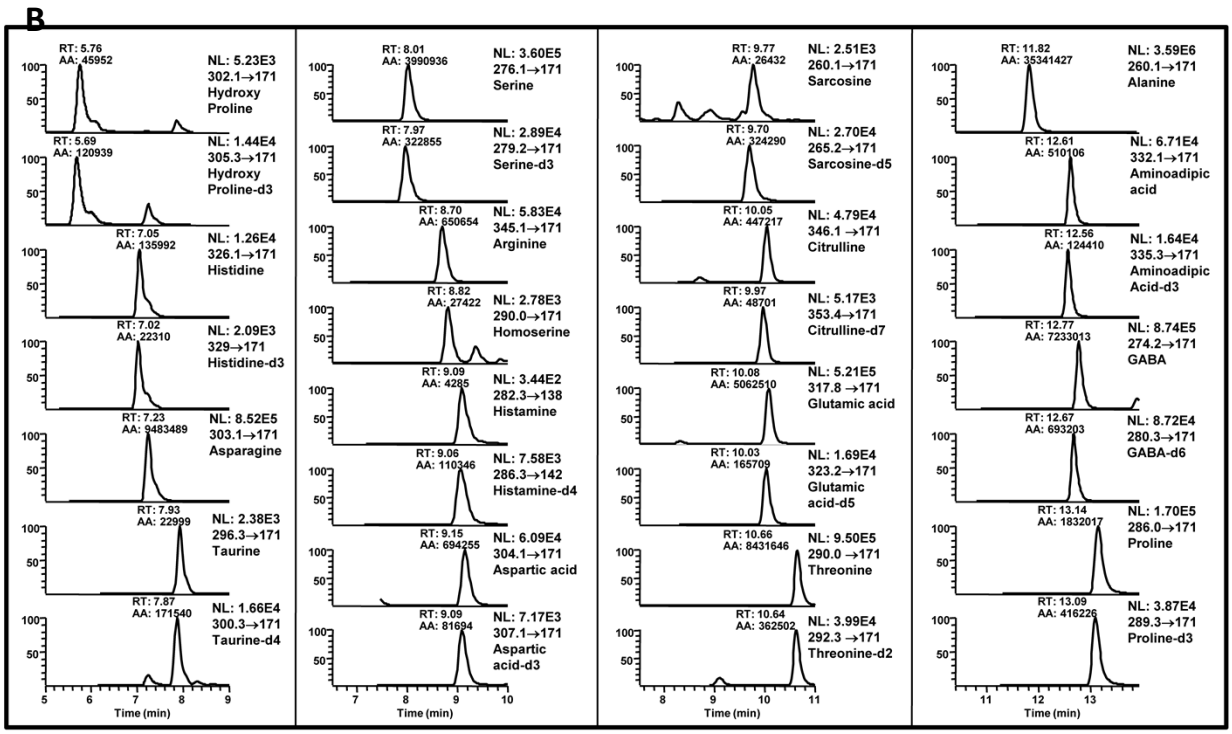



**Figure S2: UHPLC-MS/SRM chromatograms of sixteen mix amino acid.** The amino acid mix obtained from sigma was used to check the AQC reaction. All amino acids showed single sharp peak and leucine-isoleucine showed baseline separation.

**Figure S3**

**A**


B
RT: 5.76 AA: 45952
NL: 5.23E3 302.1→171 Hydroxy Proline
RT: 5.69 AA: 120939
NL: 1.44E4 305.3→171 Hydroxy Proline-d3
RT: 7.05 AA: 135992
NL: 1.26E4 326.1→171 Histidine
RT: 7.02 AA: 22310
NL: 2.09E3 329→171 Histidine-d3
RT: 7.23 AA: 9483489
NL: 8.52E5 303.1→171 Asparagine
RT: 7.93 AA: 22999
NL: 2.38E3 296.3→171 Taurine
RT: 7.87 AA: 171540
NL: 1.66E4 300.3→171 Taurine-d4
RT: 8.01 AA: 3990936
NL: 3.60E5 276.1→171 Serine
RT: 7.97 AA: 322855
NL: 2.89E4 279.2→171 Serine-d3
RT: 8.70 AA: 650654
NL: 5.83E4 345.1→171 Arginine
RT: 8.82 AA: 27422
NL: 2.78E3 290.0→171 Homoserine
RT: 9.09 AA: 4285
NL: 3.44E2 282.3→138 Histamine
RT: 9.06 AA: 110346
NL: 7.58E3 286.3→142 Histamine-d4
RT: 9.15 AA: 694255
NL: 6.09E4 304.1→171 Aspartic acid
RT: 9.09 AA: 81694
NL: 7.17E3 307.1→171 Aspartic acid-d3
RT: 9.77 AA: 26432
NL: 2.51E3 260.1→171 Sarcosine
RT: 9.70 AA: 324290
NL: 2.70E4 265.2→171 Sarcosine-d5
RT: 10.05 AA: 447217
NL: 4.79E4 346.1 →171 Citrulline
RT: 9.97 AA: 48701
NL: 5.17E3 353.4→171 Citrulline-d7
RT: 10.08 AA: 5062510
NL: 5.21E5 317.8 →171 Glutamic acid
RT: 10.03 AA: 165709
NL: 1.69E4 323.2→171 Glutamic acid-d5
RT: 10.66 AA: 8431646
NL: 9.50E5 290.0 →171 Threonine
RT: 10.64 AA: 362502
NL: 3.99E4 292.3 →171 Threonine-d2
RT: 11.82 AA: 35341427
NL: 3.59E6 260.1→171 Alanine
RT: 12.61 AA: 510106
NL: 6.71E4 332.1→171 Aminoadipic acid
RT: 12.56 AA: 124410
NL: 1.64E4 335.3→171 Aminoadipic Acid-d3
RT: 12.77 AA: 7233013
NL: 8.74E5 274.2→171 GABA
RT: 12.67 AA: 693203
NL: 8.72E4 280.3→171 GABA-d6
RT: 13.14 AA: 1832017
NL: 1.70E5 286.0→171 Proline
RT: 13.09 AA: 416226
NL: 3.87E4 289.3→171 Proline-d3
Time (min)

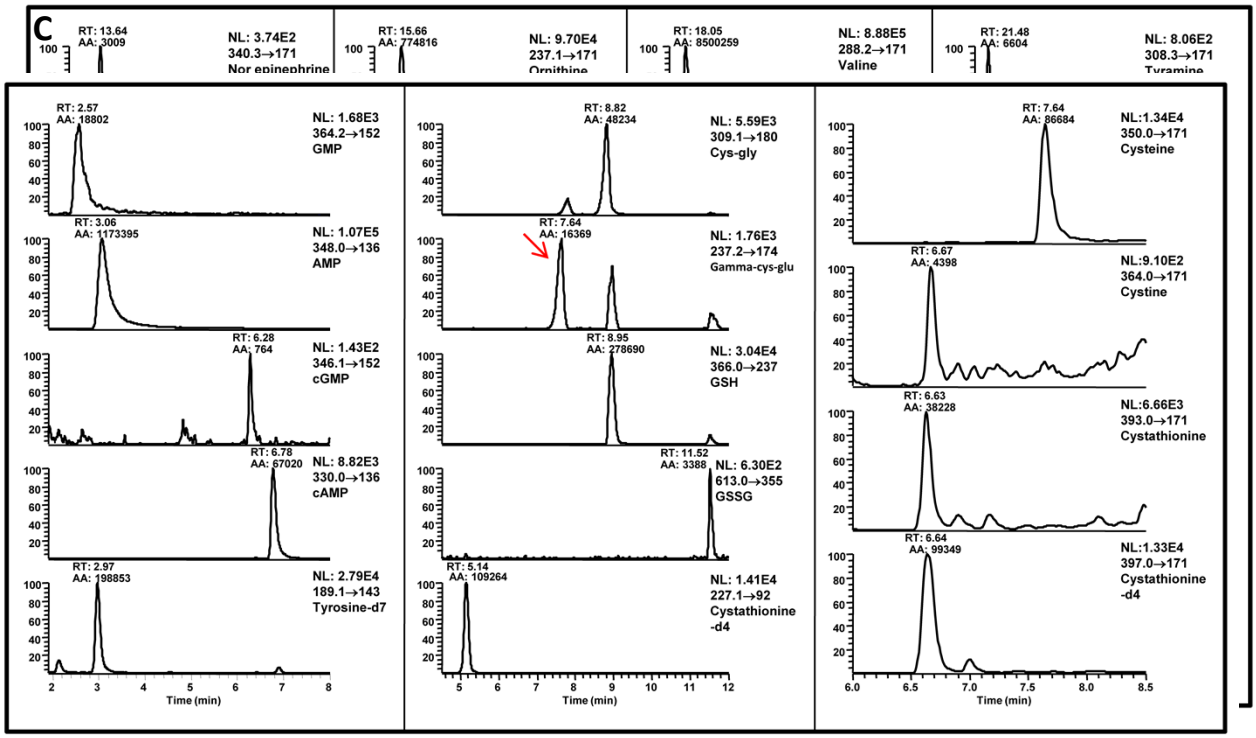

C
RT: 13.64 AA: 3009
NL: 3.74E2 340.3→171 Nor epinephrine
RT: 15.66 AA: 774816
NL: 9.70E4 237.1→171 Ornithine
RT: 18.05 AA: 8500259
NL: 8.88E5 288.2→171 Valine
RT: 21.48 AA: 6604
NL: 8.06E2 308.3→171 Tyramine
RT: 2.57 AA: 18802
NL: 1.68E3 364.2→152 GMP
RT: 3.06 AA: 1173395
NL: 1.07E5 348.0→136 AMP
RT: 6.28 AA: 764
NL: 1.43E2 346.1→152 cGMP
RT: 6.78 AA: 67020
NL: 8.82E3 330.0→136 cAMP
RT: 2.97 AA: 198853
NL: 2.79E4 189.1→143 Tyrosine-d7
RT: 8.82 AA: 48234
NL: 5.59E3 309.1→180 Cys-gly
RT: 7.64 AA: 16369
NL: 1.76E3 237.2→174 Gamma-cys-glu
RT: 8.95 AA: 278690
NL: 3.04E4 366.0→237 GSH
RT: 11.52 AA: 3388
NL: 6.30E2 613.0→355 GSSG
RT: 5.14 AA: 109264
NL: 1.41E4 227.1→92 Cystathionine -d4
RT: 7.64 AA: 86684
NL:1.34E4 350.0→171 Cysteine
RT: 6.67 AA: 4398
NL:9.10E2 364.0→171 Cystine
RT: 6.63 AA: 38228
NL:6.66E3 393.0→171 Cystathionine
RT: 6.64 AA: 99349
NL:1.33E4 397.0→171 Cystathionine -d4
Time (min)

**Figure S3: UHPLC-MS/SRM chromatogram. (**A) and (B) chromatogram of amino acid derivatives with deuterated ISTDs, (C) Nucleotide, GSH and Cysteine metabolites. The chromatogram of amines arranged based on the retention times (A and B). In (C) the arrow indicates the peak corresponding to γ-glu-cys.