# Supplementary Materials: Simultaneous Quantitation of Free Amino Acids, Nucleosides and Nucleobases in Sipunculus nudus by Ultra-High Performance Liquid Chromatography with Triple Quadrupole Mass Spectrometry

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To compare the determination results with and without internal standard, we selected adenosine cyclophosphate as internal standard since it does not exist in all the samples of the experiment.

A mixed standard stock solution containing the reference compounds 1–41 dried to constant weight was prepared in methanol/water (9:1, *v*/*v*). One 1 mL mixed standard solution was taken out and added deionized water in the same volume, and another 1 mL mixed standard solution was taken out and added internal standard solution the same volume, and a sample was treated in the same way. CA (the concentration of sample solution determined without internal standard) and CB (the concentration of sample solution determined with internal standard) were calculated by calibration curves established by one point external standard method. The T test method was applied to test the significance of difference between CA and CB, and the results indicated compared to CA, CB of most components except five (inosine, 2′-deoxyguanosine, guanine, glycine and lysine) did not show significant difference ($p > 0.05$). The details of T test showed in Table S1.

**Table S1.** T test for $C_A$ and $C_B$ of 41 components

| NO | Component | $C_A$ (mg/mL) | $C_B$ (mg/mL) | P |
|---|---|---|---|---|
| | | 0.0057 | 0.0063 | |
| 1 | Adenosine 5′-monophosphate | 0.0058 | 0.0063 | 0.2988 |
| | | 0.0056 | 0.0055 | |
| | | 0.0027 | 0.0035 | |
| 2 | Inosine | 0.0028 | 0.0034 | 0.0112 |
| | | 0.0024 | 0.0040 | |
| | | 0.0050 | 0.0041 | |
| 3 | Guanosine | 0.0044 | 0.0043 | 0.1806 |
| | | 0.0043 | 0.0043 | |
| | | 0.0212 | 0.0178 | |
| 4 | Thymidine | 0.0197 | 0.0151 | 0.0640 |
| | | 0.0177 | 0.0154 | |
| | | 0.0089 | 0.0072 | |
| 5 | 2′-deoxyuridine | 0.0076 | 0.0077 | 0.1613 |
| | | 0.0076 | 0.0066 | |
| | | 0.0230 | 0.0218 | |
| 6 | 2′-deoxyinosine | 0.0279 | 0.0250 | 0.5885 |
| | | 0.0249 | 0.0258 | |
| | | 0.0020 | 0.0020 | |
| 7 | Cytidine-5′-monophosphate | 0.0024 | 0.0020 | 0.0732 |
| | | 0.0024 | 0.0018 | |

| | | | | |
|---|---|---|---|---|
| | | 0.0048 | 0.0057 | |
| 8 | 2′-Deoxyadenosine-5′-monophosphate | 0.0054 | 0.0046 | 0.7187 |
| | | 0.0045 | 0.0048 | |
| | | 0.0096 | 0.0088 | |
| 9 | 2′-deoxycytidine | 0.0117 | 0.0100 | 0.0822 |
| | | 0.0113 | 0.0085 | |
| | | 0.3014 | 0.1875 | |
| 10 | 2′-deoxyguanosine | 0.2777 | 0.2061 | 0.0040 |
| | | 0.2568 | 0.1871 | |
| | | 0.0283 | 0.0273 | |
| 11 | Thymine | 0.0263 | 0.0257 | 0.1624 |
| | | 0.0312 | 0.0241 | |
| | | 0.0032 | 0.0031 | |
| 12 | Adenine | 0.0036 | 0.0025 | 0.1175 |
| | | 0.0031 | 0.0029 | |
| | | 0.0021 | 0.0020 | |
| 13 | Cytidine | 0.0024 | 0.0022 | 0.1076 |
| | | 0.0026 | 0.0018 | |
| | | 0.0057 | 0.0044 | |
| 14 | Uracil | 0.0062 | 0.0050 | 0.1026 |
| | | 0.0054 | 0.0054 | |
| | | 0.0505 | 0.0328 | |
| 15 | Guanine | 0.0434 | 0.0299 | 0.0029 |
| | | 0.0453 | 0.0322 | |
| | | 0.3132 | 0.3029 | |
| 16 | Xanthine | 0.2988 | 0.2843 | 0.1253 |
| | | 0.3088 | 0.2641 | |
| | | 1.1131 | 0.8857 | |
| 17 | Glycine | 1.2313 | 0.7568 | 0.0197 |
| | | 1.0125 | 0.8679 | |
| | | 0.0028 | 0.0042 | |
| 18 | GABA | 0.0034 | 0.0035 | 0.0594 |
| | | 0.0031 | 0.0037 | |
| | | 0.1451 | 0.1036 | |
| 19 | Leucine | 0.1345 | 0.1151 | 0.0787 |
| | | 0.1145 | 0.1080 | |
| | | 0.1105 | 0.0787 | |
| 20 | Isoleucine | 0.1017 | 0.0877 | 0.0517 |
| | | 0.0890 | 0.0765 | |
| | | 0.0421 | 0.0326 | |
| 21 | Methionine | 0.0380 | 0.0373 | 0.0752 |
| | | 0.0379 | 0.0303 | |
| 22 | Phenylalanine | 0.1123 | 0.0970 | 0.1190 |

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|---|---|---|---|---|
| | | 0.1235 | 0.1099 | |
| | | 0.1067 | 0.0877 | |
| | | 0.0377 | 0.0374 | |
| 23 | Tryptophan | 0.0434 | 0.0436 | 0.8021 |
| | | 0.0379 | 0.0355 | |
| | | 0.5245 | 0.4556 | |
| 24 | Alanine | 0.4234 | 0.5038 | 0.8022 |
| | | 0.4766 | 0.4368 | |
| | | 0.1456 | 0.1215 | |
| 25 | Threonine | 0.1320 | 0.1110 | 0.1523 |
| | | 0.1277 | 0.1310 | |
| | | 0.2566 | 0.1914 | |
| 26 | Serine | 0.2200 | 0.2099 | 0.0723 |
| | | 0.2172 | 0.1872 | |
| | | 0.1516 | 0.1303 | |
| 27 | Asparagine | 0.1365 | 0.1102 | 0.1239 |
| | | 0.1270 | 0.1208 | |
| | | 0.0842 | 0.0728 | |
| 28 | Glutamine | 0.0704 | 0.0658 | 0.2791 |
| | | 0.0694 | 0.0654 | |
| | | 0.4038 | 0.3484 | |
| 29 | Glutamate | 0.3715 | 0.3271 | 0.0888 |
| | | 0.3460 | 0.3026 | |
| | | 0.0652 | 0.0439 | |
| 30 | Citrulline | 0.0560 | 0.0508 | 0.1203 |
| | | 0.0547 | 0.0544 | |
| | | 0.1045 | 0.0979 | |
| 31 | Proline | 0.1123 | 0.0997 | 0.1493 |
| | | 0.1234 | 0.1086 | |
| | | 0.0182 | 0.0164 | |
| 32 | Hydroxyproline | 0.0214 | 0.0165 | 0.0558 |
| | | 0.0189 | 0.0175 | |
| | | 0.0134 | 0.0121 | |
| 33 | Cysteine | 0.0142 | 0.0111 | 0.0989 |
| | | 0.0121 | 0.0121 | |
| | | 0.4657 | 0.5131 | |
| 34 | Tyrosine | 0.5454 | 0.5245 | 0.5467 |
| | | 0.4879 | 0.5090 | |
| | | 1.2324 | 1.6566 | |
| 35 | Taurine | 1.3435 | 1.4457 | 0.0639 |
| | | 1.3453 | 1.4365 | |
| 36 | Valine | 0.3345 | 0.3425 | 0.0732 |
| | | 0.2789 | 0.3979 | |

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|---|---|---|---|---|
| | | 0.2990 | 0.3468 | |
| | | 0.0165 | 0.0184 | |
| 37 | Ornithine | 0.0165 | 0.0184 | 0.0588 |
| | | 0.0175 | 0.0209 | |
| | | 0.2148 | 0.1919 | |
| 38 | Aspartate | 0.1820 | 0.1645 | 0.1056 |
| | | 0.2083 | 0.1623 | |
| | | 0.3778 | 0.2739 | |
| 39 | Lysine | 0.3302 | 0.2919 | 0.0236 |
| | | 0.3298 | 0.2428 | |
| | | 0.0410 | 0.0466 | |
| 40 | Histidine | 0.0450 | 0.0480 | 0.9632 |
| | | 0.0493 | 0.0412 | |
| | | 0.5702 | 0.4646 | |
| 41 | Arginine | 0.5256 | 0.4383 | 0.0766 |
| | | 0.4669 | 0.4376 | |