

# **Analysis of Total Human Urinary Glycosaminoglycan Disaccharides by Liquid Chromatography-Tandem Mass Spectrometry**

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## Table of Contents

|                                                                                                                                                                                                                                         |      |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| <b>Title page</b> .....                                                                                                                                                                                                                 | S-1  |
| <b>Table of Contents</b> .....                                                                                                                                                                                                          | S-2  |
| <b>Table S1</b> HS, CS content and composition and HA content of trauma patients urine samples (ng/mL).....                                                                                                                             | S-3  |
| <b>Table S2</b> HS, CS content and composition and HA content of trauma patients urine samples (ng/mg creatinine).....                                                                                                                  | S-4  |
| <b>Table S3</b> DMMB assay of GAG content of trauma patient's urine samples and normal human urine samples in $\mu\text{g/mL}$ and $\mu\text{g/mg}$ creatinine .....                                                                    | S-5  |
| <b>Table S4</b> HS, CS content and composition and HA content for normal human urine samples (ng/mL).....                                                                                                                               | S-6  |
| <b>Table S5</b> HS, CS content and composition and HA content normal human urine samples (ng/mg creatinine).....                                                                                                                        | S-7  |
| <b>Figure S1.</b> The GAG content of pooled normal human urine sample using single tube preparation and conventional preparation.....                                                                                                   | S-8  |
| <b>Figure S2.</b> The kinetic study on AMAC labeling.....                                                                                                                                                                               | S-9  |
| <b>Figure S3.</b> The average percentage composition of urinary GAGs, HS, CS and HA and the disaccharide composition of urinary HS and CS of normal human urine samples.....                                                            | S-10 |
| <b>Figure S4.</b> DMMB assay and its correlation to the LC-MS/MS assay. A. Standard curve for DMMB assay absorbance at 525 nm is plotted as a function of concentration ( $\mu\text{g/mL}$ ). B. Correlation to the LC-MS/MS assay..... | S-11 |

Table S1 HS, CS content and composition and HA content of trauma patients urine samples (ng/mL)

| Study ID | TOTAL GAG (ng/mL) |      |     |       | CS (ng/mL) |      |      |      |    |      |     |      | HS (ng/mL) |      |      |     |      |     |     |     |
|----------|-------------------|------|-----|-------|------------|------|------|------|----|------|-----|------|------------|------|------|-----|------|-----|-----|-----|
|          | HS                | CS   | HA  | TOTAL | TriS       | 2S4S | 2S6S | 4S6S | 2S | 4S   | 6S  | 0S   | TriS       | NS6S | NS2S | NS  | 2S6S | 6S  | 2S  | 0S  |
| 002-0001 | 1141              | 5402 | 408 | 6951  | 3.3        | 18   | 34   | 104  | ND | 2780 | 413 | 2050 | 60         | 49   | 118  | 316 | ND   | 64  | 32  | 501 |
| 002-0002 | 495               | 2259 | 229 | 2983  | 1.0        | 5.0  | 8.9  | 28   | ND | 492  | 134 | 1591 | 31         | 18   | 51   | 136 | ND   | 28  | 8.2 | 223 |
| 002-0003 | 704               | 7323 | 583 | 8610  | 2.4        | 15   | 25   | 58   | ND | 3986 | 363 | 2873 | 28         | 20   | 62   | 159 | ND   | 34  | 20  | 380 |
| 002-0004 | 1891              | 6446 | 643 | 8980  | 3.3        | 22   | 42   | 97   | ND | 2903 | 776 | 2604 | 82         | 64   | 186  | 567 | 3.4  | 114 | 42  | 833 |
| 002-0006 | 417               | 6583 | 142 | 7143  | 2.7        | 14   | 24   | 83   | ND | 3705 | 766 | 1988 | 14         | 15   | 39   | 92  | ND   | 21  | 10  | 226 |
| 002-0007 | 1157              | 5716 | 386 | 7259  | 6.2        | 22   | 24   | 148  | ND | 2999 | 497 | 2020 | 53         | 42   | 116  | 313 | 4.7  | 68  | 24  | 537 |
| 002-0009 | 420               | 1287 | 68  | 1775  | 0.2        | 5.7  | 5.2  | 20   | ND | 708  | 93  | 456  | 15         | 18   | 58   | 103 | ND   | 27  | 4.7 | 194 |
| 002-0010 | 195               | 1915 | 57  | 2167  | 3.1        | 6.2  | 11   | 36   | ND | 1042 | 133 | 684  | 4.3        | 6.4  | 21   | 43  | 1.2  | 10  | 2.7 | 106 |
| 002-0011 | 669               | 4474 | 196 | 5339  | 5.4        | 22   | 37   | 95   | ND | 2245 | 740 | 1331 | 16         | 21   | 68   | 158 | 2.4  | 34  | 12  | 359 |
| 002-0012 | 858               | 4867 | 319 | 6044  | 2.8        | 16   | 24   | 74   | ND | 2681 | 346 | 1724 | 22         | 25   | 99   | 203 | 3.1  | 54  | 19  | 433 |
| 002-0013 | 184               | 888  | 131 | 1203  | 1.3        | 6.5  | 10   | 30   | ND | 350  | 112 | 378  | 7.5        | 6.8  | 17   | 43  | 0.8  | 8.7 | 4.9 | 96  |
| 002-0014 | 1127              | 4905 | 307 | 6339  | 3.5        | 15   | 33   | 108  | ND | 2581 | 630 | 1536 | 59         | 47   | 118  | 288 | 3.8  | 65  | 23  | 523 |
| 002-0015 | 610               | 3536 | 192 | 4337  | 2.2        | 11   | 24   | 71   | ND | 2324 | 362 | 740  | 38         | 24   | 58   | 171 | 1.9  | 37  | 8   | 272 |
| 002-0016 | 508               | 3161 | 162 | 3831  | 2.9        | 14   | 35   | 62   | ND | 1377 | 566 | 1106 | 29         | 22   | 45   | 129 | 2.0  | 28  | 10  | 243 |
| 002-0018 | 932               | 5797 | 156 | 6884  | 3.5        | 18   | 24   | 77   | ND | 3780 | 614 | 1281 | 35         | 29   | 121  | 208 | 3.0  | 58  | 20  | 457 |
| 002-0019 | 145               | 916  | 56  | 1117  | 3.2        | 2.5  | 7.5  | 32   | ND | 415  | 138 | 319  | 0.5        | 2.5  | 14   | 26  | 0.7  | 8.3 | 1.1 | 92  |
| 002-0021 | 2111              | 4108 | 310 | 6529  | 4.4        | 24   | 38   | 73   | ND | 2195 | 636 | 1138 | 130        | 79   | 226  | 649 | 6.1  | 119 | 46  | 857 |
| 002-0022 | 1102              | 5278 | 783 | 7163  | 3.4        | 18   | 28   | 73   | ND | 2566 | 352 | 2238 | 35         | 36   | 135  | 262 | 2.8  | 72  | 25  | 535 |
| 002-0024 | 220               | 316  | 45  | 580   | 0.1        | 2.6  | 3.2  | 7.1  | ND | 103  | 26  | 173  | 11         | 8.2  | 20   | 58  | 0.6  | 12  | 3.0 | 107 |
| 002-0025 | 188               | 1364 | 171 | 1723  | 0.4        | 6.4  | 5.1  | 20   | ND | 655  | 40  | 637  | 5.5        | 5.4  | 17   | 41  | 0.3  | 8.7 | 4.5 | 105 |
| 002-0026 | 794               | 4578 | 393 | 5765  | 2.4        | 15   | 21   | 55   | ND | 2317 | 400 | 1767 | 24         | 24   | 82   | 192 | 1.9  | 46  | 22  | 402 |
| 002-0027 | 1474              | 9287 | 438 | 11198 | 5.5        | 33   | 35   | 160  | ND | 7412 | 297 | 1345 | 77         | 52   | 158  | 381 | 4.7  | 80  | 46  | 675 |
| 002-0029 | 1072              | 2729 | 704 | 4504  | 4.9        | 20   | 29   | 87   | ND | 1637 | 511 | 440  | 55         | 42   | 121  | 256 | 3.3  | 64  | 34  | 496 |
| Average  | 801               | 4049 | 299 | 5149  | 3          | 14   | 23   | 69   | ND | 2228 | 389 | 1322 | 36         | 29   | 85   | 208 | 3    | 46  | 18  | 376 |

Table S2. HS, CS content and composition and HA content of trauma patients urine samples (ng/mg creatinine)

| Study ID | TOTAL GAG (ng/mg creatinine) |      |     |       | CS (ng/mg creatinine) |      |      |      |    |      |     |      | HS (ng/mg creatinine) |      |      |     |      |     |     |      |
|----------|------------------------------|------|-----|-------|-----------------------|------|------|------|----|------|-----|------|-----------------------|------|------|-----|------|-----|-----|------|
|          | HS                           | CS   | HA  | TOTAL | TriS                  | 2S4S | 2S6S | 4S6S | 2S | 4S   | 6S  | 0S   | TriS                  | NS6S | NS2S | NS  | 2S6S | 6S  | 2S  | 0S   |
| 002-0001 | 1047                         | 4956 | 374 | 6377  | 3.0                   | 17   | 31   | 96   | ND | 2550 | 379 | 1881 | 55                    | 45   | 109  | 290 | ND   | 59  | 29  | 459  |
| 002-0002 | 1793                         | 8186 | 829 | 10808 | 3.8                   | 18   | 32   | 102  | ND | 1783 | 484 | 5764 | 112                   | 66   | 183  | 493 | ND   | 101 | 30  | 808  |
| 002-0003 | 435                          | 4526 | 360 | 5322  | 1.5                   | 9    | 15   | 36   | ND | 2464 | 225 | 1776 | 18                    | 13   | 38   | 99  | ND   | 21  | 12  | 235  |
| 002-0004 | 1014                         | 3456 | 345 | 4815  | 1.8                   | 12   | 22   | 52   | ND | 1556 | 416 | 1396 | 44                    | 34   | 100  | 304 | 2.0  | 61  | 23  | 447  |
| 002-0006 | 459                          | 7242 | 157 | 7858  | 3.0                   | 16   | 27   | 91   | ND | 4075 | 843 | 2187 | 15                    | 17   | 43   | 101 | ND   | 23  | 11  | 248  |
| 002-0007 | 1327                         | 6555 | 442 | 8325  | 7.1                   | 25   | 28   | 170  | ND | 3439 | 570 | 2317 | 60                    | 48   | 133  | 359 | 5.0  | 78  | 28  | 616  |
| 002-0009 | 2283                         | 6996 | 369 | 9649  | 1.4                   | 31   | 28   | 109  | ND | 3848 | 503 | 2476 | 79                    | 100  | 317  | 561 | ND   | 145 | 26  | 1056 |
| 002-0010 | 470                          | 4626 | 139 | 5235  | 7.5                   | 15   | 26   | 86   | ND | 2517 | 322 | 1651 | 10                    | 15   | 50   | 105 | 3.0  | 24  | 6.0 | 256  |
| 002-0011 | 448                          | 3001 | 131 | 3581  | 3.6                   | 15   | 25   | 63   | ND | 1506 | 496 | 893  | 10                    | 14   | 46   | 106 | 2.0  | 23  | 8.0 | 241  |
| 002-0012 | 828                          | 4698 | 307 | 5834  | 2.7                   | 15   | 23   | 71   | ND | 2588 | 334 | 1664 | 21                    | 24   | 96   | 196 | 3.0  | 52  | 18  | 418  |
| 002-0013 | 636                          | 3072 | 454 | 4162  | 4.5                   | 22   | 34   | 103  | ND | 1212 | 387 | 1309 | 26                    | 24   | 58   | 148 | 3.0  | 30  | 17  | 330  |
| 002-0014 | 642                          | 2797 | 175 | 3614  | 2.0                   | 8.3  | 19   | 62   | ND | 1471 | 359 | 876  | 34                    | 27   | 67   | 164 | 2.0  | 37  | 13  | 298  |
| 002-0015 | 880                          | 5102 | 277 | 6259  | 3.2                   | 16   | 35   | 103  | ND | 3354 | 523 | 1068 | 54                    | 35   | 83   | 247 | 3.0  | 53  | 12  | 392  |
| 002-0016 | 326                          | 2032 | 104 | 2462  | 1.8                   | 9.0  | 23   | 40   | ND | 885  | 364 | 711  | 18                    | 14   | 29   | 83  | 1.0  | 18  | 6.0 | 156  |
| 002-0018 | 828                          | 5152 | 138 | 6119  | 3.1                   | 16   | 22   | 69   | ND | 3360 | 546 | 1138 | 31                    | 26   | 108  | 185 | 3.0  | 52  | 17  | 406  |
| 002-0019 | 186                          | 1173 | 71  | 1430  | 4.0                   | 3.2  | 10   | 41   | ND | 531  | 177 | 408  | 1.0                   | 3.0  | 18   | 34  | 1.0  | 11  | 1.0 | 117  |
| 002-0021 | 986                          | 1918 | 145 | 3048  | 2.1                   | 11   | 18   | 34   | ND | 1025 | 297 | 531  | 61                    | 37   | 105  | 303 | 3.0  | 56  | 21  | 400  |
| 002-0022 | 841                          | 4029 | 597 | 5468  | 2.6                   | 14   | 21   | 56   | ND | 1958 | 269 | 1708 | 26                    | 27   | 103  | 200 | 2.0  | 55  | 19  | 408  |
| 002-0024 | 810                          | 1165 | 164 | 2139  | 0.4                   | 10   | 12   | 26   | ND | 380  | 97  | 640  | 41                    | 30   | 74   | 213 | 2.0  | 43  | 11  | 395  |
| 002-0025 | 1011                         | 7331 | 922 | 9265  | 2.1                   | 35   | 27   | 107  | ND | 3523 | 216 | 3422 | 30                    | 29   | 91   | 223 | 2.0  | 47  | 24  | 566  |
| 002-0026 | 916                          | 5280 | 454 | 6650  | 2.8                   | 18   | 24   | 64   | ND | 2672 | 462 | 2038 | 27                    | 28   | 94   | 221 | 2.0  | 54  | 26  | 463  |
| 002-0027 | 1361                         | 8575 | 404 | 10340 | 5.1                   | 30   | 33   | 147  | ND | 6844 | 274 | 1242 | 71                    | 48   | 145  | 351 | 4.0  | 74  | 43  | 624  |
| 002-0029 | 1206                         | 3069 | 792 | 5067  | 5.5                   | 23   | 33   | 98   | ND | 1841 | 574 | 495  | 62                    | 47   | 136  | 288 | 4.0  | 72  | 38  | 558  |
| Average  | 901                          | 4563 | 354 | 5818  | 3                     | 17   | 25   | 79   | ND | 2408 | 396 | 1634 | 39                    | 33   | 97   | 229 | 3    | 52  | 19  | 430  |

Table S3. DMMB assay of GAG content of trauma patients urine samples and normal human urine samples in  $\mu\text{g/mL}$  and ( $\mu\text{g/mg}$  creatinine)

| <b>GAG content of trauma patients urine samples</b> |                                                              |                                                             |                                                                            |
|-----------------------------------------------------|--------------------------------------------------------------|-------------------------------------------------------------|----------------------------------------------------------------------------|
| <b>Sample ID</b>                                    | <b>Average GAG Content<br/>(<math>\mu\text{g/mL}</math>)</b> | <b>Standard deviation<br/>(<math>\mu\text{g/mL}</math>)</b> | <b>Normalized GAG Content<br/>(<math>\mu\text{g/mg}</math> creatinine)</b> |
| 002-0001                                            | 21                                                           | 1                                                           | 19                                                                         |
| 002-0002                                            | 4                                                            | 3                                                           | 13                                                                         |
| 002-0003                                            | 46                                                           | 5                                                           | 29                                                                         |
| 002-0004                                            | 23                                                           | 3                                                           | 12                                                                         |
| 002-0006                                            | 20                                                           | 1                                                           | 22                                                                         |
| 002-0007                                            | 28                                                           | 4                                                           | 32                                                                         |
| 002-0009                                            | 5                                                            | 2                                                           | 25                                                                         |
| 002-0010                                            | 3                                                            | 2                                                           | 7                                                                          |
| 002-0011                                            | 14                                                           | 4                                                           | 9                                                                          |
| 002-0012                                            | 17                                                           | 9                                                           | 16                                                                         |
| 002-0013                                            | 3                                                            | 2                                                           | 12                                                                         |
| 002-0014                                            | 46                                                           | 2                                                           | 26                                                                         |
| 002-0015                                            | 11                                                           | 2                                                           | 15                                                                         |
| 002-0016                                            | 8                                                            | 5                                                           | 5                                                                          |
| 002-0018                                            | 25                                                           | 5                                                           | 22                                                                         |
| 002-0019                                            | 1                                                            | 1                                                           | 1                                                                          |
| 002-0021                                            | 31                                                           | 3                                                           | 15                                                                         |
| 002-0022                                            | 24                                                           | 8                                                           | 18                                                                         |
| 002-0024                                            | 3                                                            | 2                                                           | 11                                                                         |
| 002-0025                                            | 4                                                            | 4                                                           | 21                                                                         |
| 002-0026                                            | 18                                                           | 12                                                          | 21                                                                         |
| 002-0027                                            | 40                                                           | 7                                                           | 37                                                                         |
| 002-0029                                            | 20                                                           | 3                                                           | 22                                                                         |
| Average                                             | 18                                                           | 4                                                           | 18                                                                         |
| <b>GAG content of normal human urine samples</b>    |                                                              |                                                             |                                                                            |
| <b>Sample ID</b>                                    | <b>Average GAG Content<br/>(<math>\mu\text{g/mL}</math>)</b> | <b>Standard deviation<br/>(<math>\mu\text{g/mL}</math>)</b> | <b>Normalized GAG Content<br/>(<math>\mu\text{g/mg}</math> creatinine)</b> |
| Pooled urine                                        | 18                                                           | 3                                                           | 16                                                                         |
| Male Urine 1                                        | 4                                                            | 2                                                           | 3                                                                          |
| Male Urine 2                                        | 9                                                            | 3                                                           | 7                                                                          |
| Female Urine 1                                      | 13                                                           | 9                                                           | 13                                                                         |
| Female Urine 2                                      | 16                                                           | 4                                                           | 14                                                                         |
| Average                                             | 12                                                           | 4                                                           | 17                                                                         |

Table S4 HS, CS content and composition and HA content for normal human urine samples (ng/mL)

| Study ID       | TOTAL GAG (ng/mL) |      |     |       | HS (ng/mL) |      |      |     |      |     |    |      | CS (ng/mL) |      |      |      |    |      |      |     |
|----------------|-------------------|------|-----|-------|------------|------|------|-----|------|-----|----|------|------------|------|------|------|----|------|------|-----|
|                | HS                | CS   | HA  | TOTAL | TriS       | NS6S | NS2S | NS  | 2S6S | 6S  | 2S | 0S   | TriS       | 2S4S | 4S6S | 2S6S | 2S | 4S   | 6S   | 0S  |
| Pooled Urine   | 1257              | 2491 | 258 | 4007  | 88         | 83   | 139  | 239 | ND   | 159 | 36 | 513  | 1.4        | 29   | 46   | 111  | ND | 1355 | 832  | 116 |
| Male Urine 1   | 876               | 2902 | 239 | 4017  | 37         | 46   | 95   | 189 | ND   | 85  | 15 | 409  | 1.5        | 24   | 59   | 73   | ND | 1409 | 874  | 461 |
| Male Urine 2   | 2334              | 3742 | 307 | 6383  | 151        | 156  | 227  | 353 | ND   | 313 | 72 | 1063 | 7.1        | 69   | 60   | 180  | ND | 2221 | 898  | 308 |
| Female Urine 1 | 1762              | 3151 | 296 | 5209  | 100        | 95   | 173  | 300 | ND   | 216 | 50 | 828  | 5.2        | 52   | 74   | 148  | ND | 2025 | 699  | 148 |
| Female Urine 2 | 2394              | 5654 | 63  | 8110  | 117        | 119  | 242  | 464 | ND   | 272 | 34 | 117  | 7.2        | 61   | 87   | 202  | ND | 3851 | 1099 | 347 |

Table S5. HS, CS content and composition and HA content normal human urinesamples (ng/mg creatinine)

| Study ID       | TOTAL GAG (ng/mg creatinine) |      |     |       | HS (ng/mg creatinine) |      |      |     |      |     |    |      | CS (ng/mg creatinine) |      |      |      |    |      |     |     |
|----------------|------------------------------|------|-----|-------|-----------------------|------|------|-----|------|-----|----|------|-----------------------|------|------|------|----|------|-----|-----|
|                | HS                           | CS   | HA  | TOTAL | TriS                  | NS6S | NS2S | NS  | 2S6S | 6S  | 2S | 0S   | TriS                  | 2S4S | 4S6S | 2S6S | 2S | 4S   | 6S  | 0S  |
| Pooled Urine   | 1122                         | 2224 | 231 | 3577  | 78                    | 74   | 124  | 214 | ND   | 142 | 32 | 458  | 1.3                   | 26   | 41   | 99   | ND | 1210 | 743 | 104 |
| Male Urine 1   | 811                          | 2687 | 221 | 3719  | 34                    | 43   | 88   | 175 | ND   | 79  | 14 | 379  | 1.4                   | 22   | 55   | 67   | ND | 1305 | 809 | 427 |
| Male Urine 2   | 1898                         | 3042 | 249 | 5190  | 123                   | 127  | 185  | 287 | ND   | 254 | 59 | 864  | 5.7                   | 56   | 49   | 146  | ND | 1806 | 730 | 250 |
| Female Urine 1 | 1836                         | 3282 | 308 | 5426  | 105                   | 99   | 180  | 313 | ND   | 225 | 52 | 863  | 5.4                   | 55   | 77   | 154  | ND | 2109 | 728 | 154 |
| Female Urine 2 | 2119                         | 5003 | 492 | 7614  | 104                   | 105  | 214  | 411 | ND   | 241 | 30 | 1014 | 6.3                   | 54   | 77   | 178  | ND | 3408 | 972 | 307 |

Figure S1

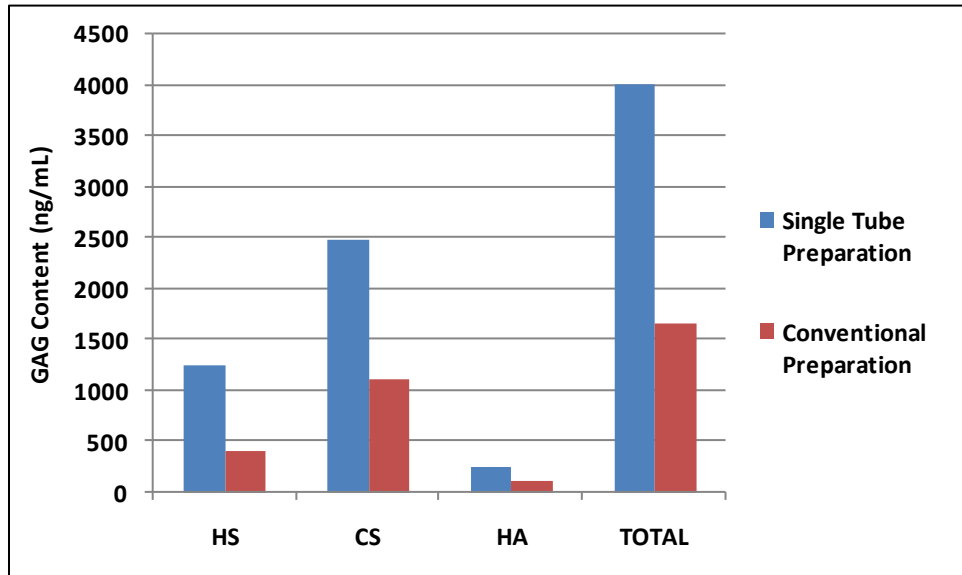




Figure S2

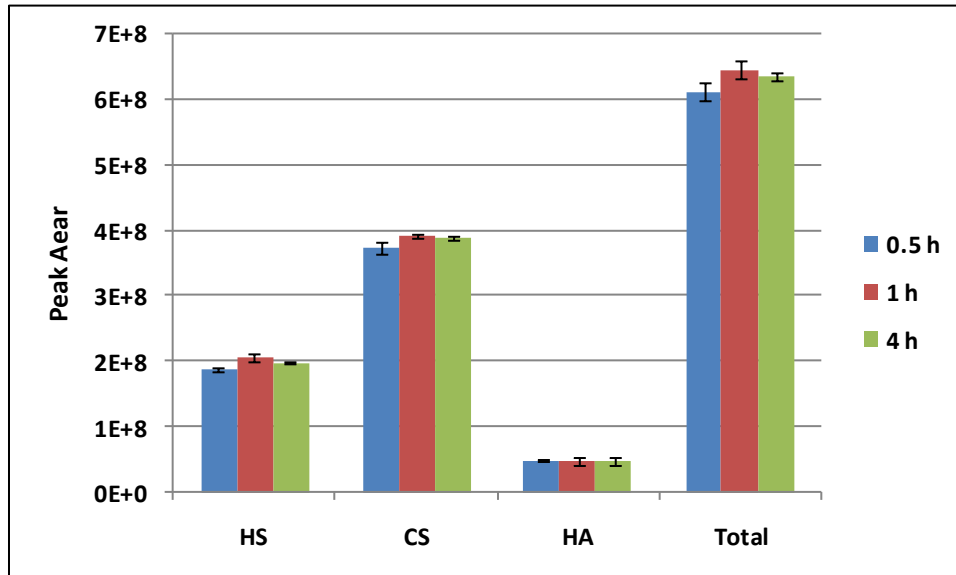


Figure S3

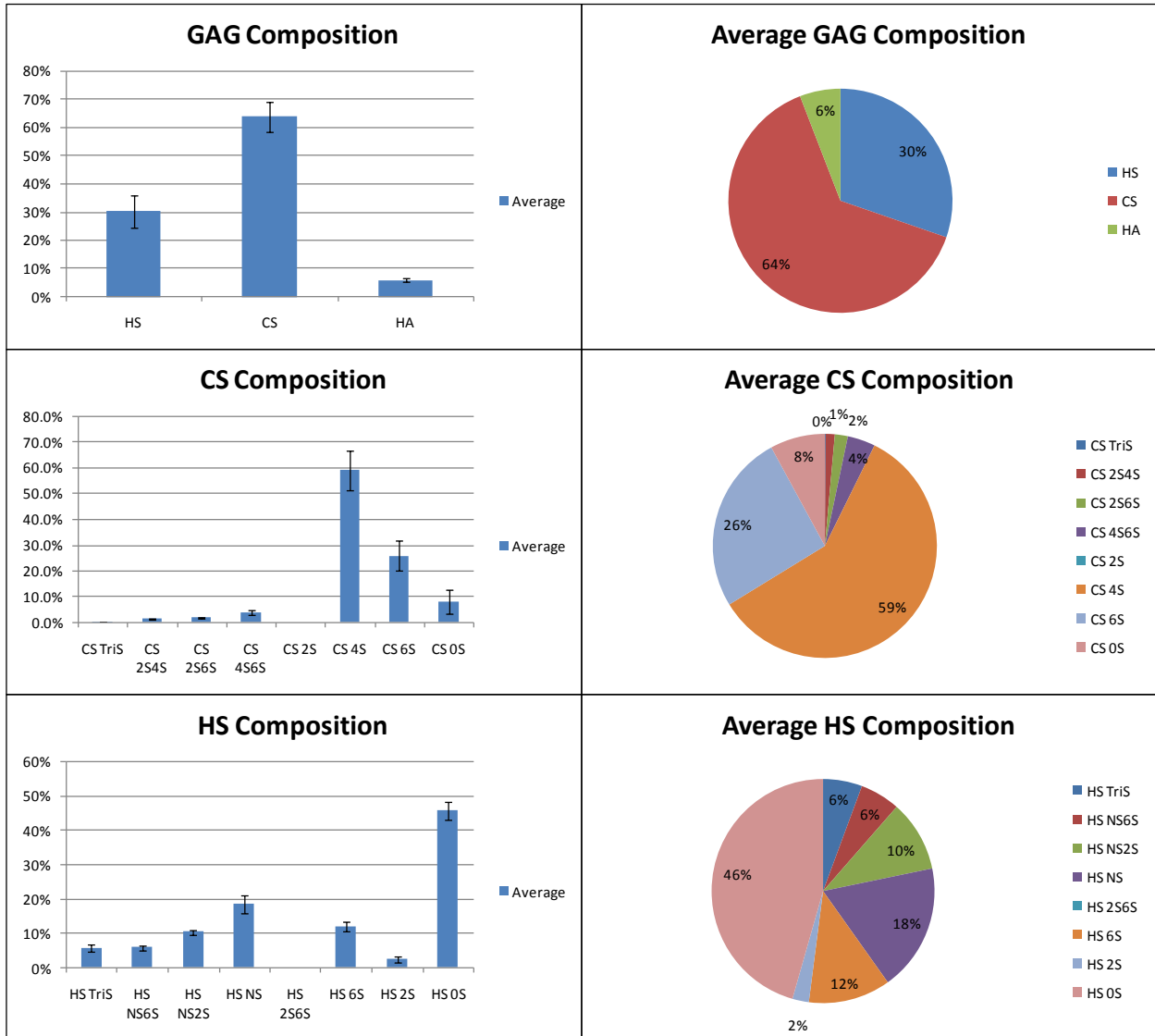
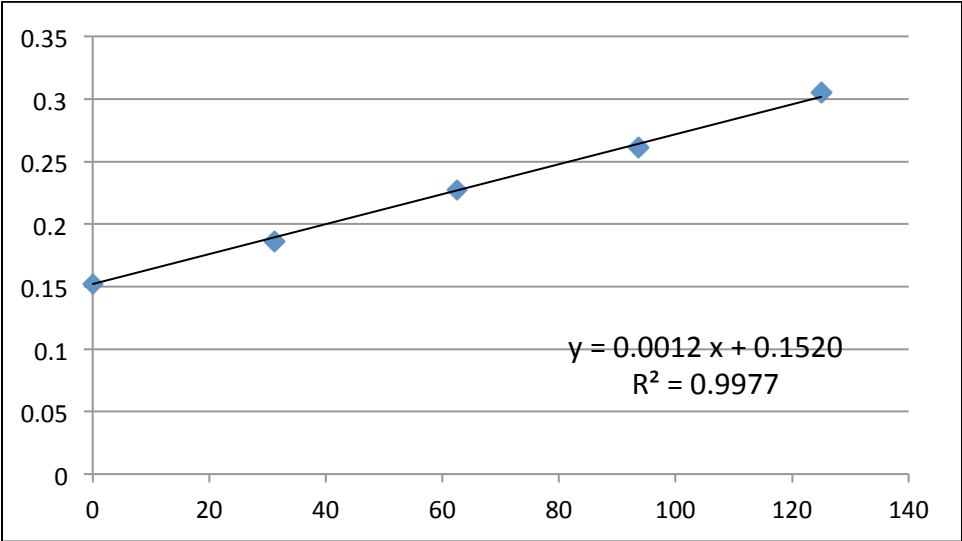


Figure S4

A



B

