## Supplementary Material

# Analysis of Derivatized N-Acyl Amino Acid Surfactants using HPLC and HPLC/MS

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**Table S1.** Signals for protonated molecules $[M+H]^+$ of native and derivatized N-acyl-glutamate surfactants (CS-11, CS-22, CT-12S, ECS-22SB) with different fatty acid moieties; BPAE = 4′-bromophenacyl esters of surfactants, diBPAE = corresponding diesters.

| Fatty acid | Native surfactant | | BPAE | | diBPAE | |
|---|---|---|---|---|---|---|
| | Formula | $[M+H]^+$ | Formula | $[M+H]^+$ | Formula | $[M+H]^+$ |
| Caprylic, C8:0 | $C_{13}H_{24}O_5N^+$ | 274.2 | $C_{21}H_{29}O_6NBr^+$ | 470.1 | $C_{29}H_{34}O_7NBr_2^+$ | 666.1 |
| Capric, C10:0 | $C_{15}H_{28}O_5N^+$ | 302.2 | $C_{23}H_{33}O_6NBr^+$ | 498.1 | $C_{31}H_{38}O_7NBr_2^+$ | 694.1 |
| Lauric, C12:0 | $C_{17}H_{32}O_5N^+$ | 330.2 | $C_{25}H_{37}O_6NBr^+$ | 526.2 | $C_{33}H_{42}O_7NBr_2^+$ | 722.1 |
| Myristic, C14:0 | $C_{19}H_{36}O_5N^+$ | 358.3 | $C_{27}H_{41}O_6NBr^+$ | 554.2 | $C_{35}H_{46}O_7NBr_2^+$ | 750.2 |
| Palmitic, C16:0 | $C_{21}H_{40}O_5N^+$ | 386.3 | $C_{29}H_{45}O_6NBr^+$ | 582.2 | $C_{37}H_{50}O_7NBr_2^+$ | 778.2 |
| Oleic, C18:1 | $C_{23}H_{42}O_5N^+$ | 412.3 | $C_{31}H_{47}O_6NBr^+$ | 608.3 | $C_{39}H_{52}O_7NBr_2^+$ | 804.2 |

**Table S2.** Signals for protonated molecules $[M+H]^+$ of native and derivatized N-acyl-threoninate surfactant (ET-CS-12) with different fatty acid moieties; BPAE = 4′-bromophenacyl esters of surfactant.

| Fatty acid | Native surfactant | | BPAE | |
|---|---|---|---|---|
| | Formula | $[M+H]^+$ | Formula | $[M+H]^+$ |
| Caprylic, C8:0 | $C_{12}H_{24}O_4N^+$ | 246.2 | $C_{20}H_{29}O_5NBr^+$ | 442.1 |
| Capric, C10:0 | $C_{14}H_{28}O_4N^+$ | 274.2 | $C_{22}H_{33}O_5NBr^+$ | 470.2 |
| Lauric, C12:0 | $C_{16}H_{32}O_4N^+$ | 302.2 | $C_{24}H_{37}O_5NBr^+$ | 498.2 |
| Myristic, C14:0 | $C_{18}H_{36}O_4N^+$ | 330.3 | $C_{26}H_{41}O_5NBr^+$ | 526.2 |
| Palmitic, C16:0 | $C_{20}H_{40}O_4N^+$ | 358.3 | $C_{28}H_{45}O_5NBr^+$ | 554.2 |
| Oleic, C18:1 | $C_{22}H_{42}O_4N^+$ | 384.3 | $C_{30}H_{47}O_5NBr^+$ | 580.3 |

**Table S3.** Signals for protonated molecules $[M+H]^+$ of native and derivatized N-acyl-glycine surfactants (GCK-12H, GCS-12K) with different fatty acid moieties; BPAE = 4′-bromophenacyl esters of surfactants.

| Fatty acid | Native surfactant | | BPAE | |
|---|---|---|---|---|
| | Formula | $[M+H]^+$ | Formula | $[M+H]^+$ |
| Caprylic, C8:0 | $C_{10}H_{20}O_3N^+$ | 202.1 | $C_{18}H_{25}O_4NBr^+$ | 398.2 |
| Capric, C10:0 | $C_{12}H_{24}O_3N^+$ | 230.2 | $C_{20}H_{29}O_4NBr^+$ | 426.1 |
| Lauric, C12:0 | $C_{14}H_{28}O_3N^+$ | 258.2 | $C_{22}H_{33}O_4NBr^+$ | 454.2 |
| Myristic, C14:0 | $C_{16}H_{32}O_3N^+$ | 287.2 | $C_{24}H_{37}O_4NBr^+$ | 482.2 |
| Palmitic, C16:0 | $C_{18}H_{36}O_3N^+$ | 314.3 | $C_{26}H_{41}O_4NBr^+$ | 510.2 |
| Oleic, C18:1 | $C_{20}H_{38}O_3N^+$ | 340.3 | $C_{28}H_{43}O_4NBr^+$ | 536.2 |

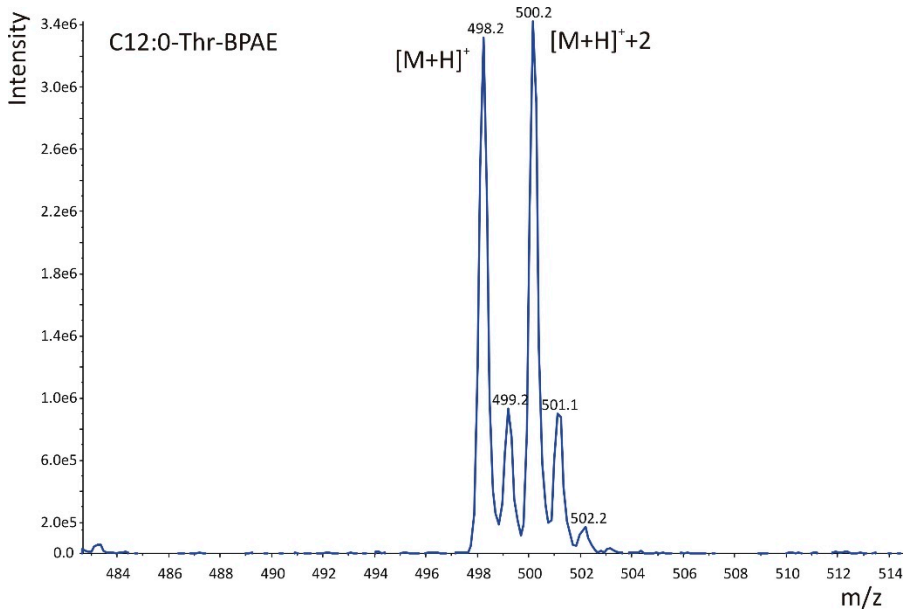


**Figure S1.** Isotopic pattern of protonated molecule of the 4′-bromophenacyl ester derivative of N-lauryl-threoninate surfactant determined using LC/MS.

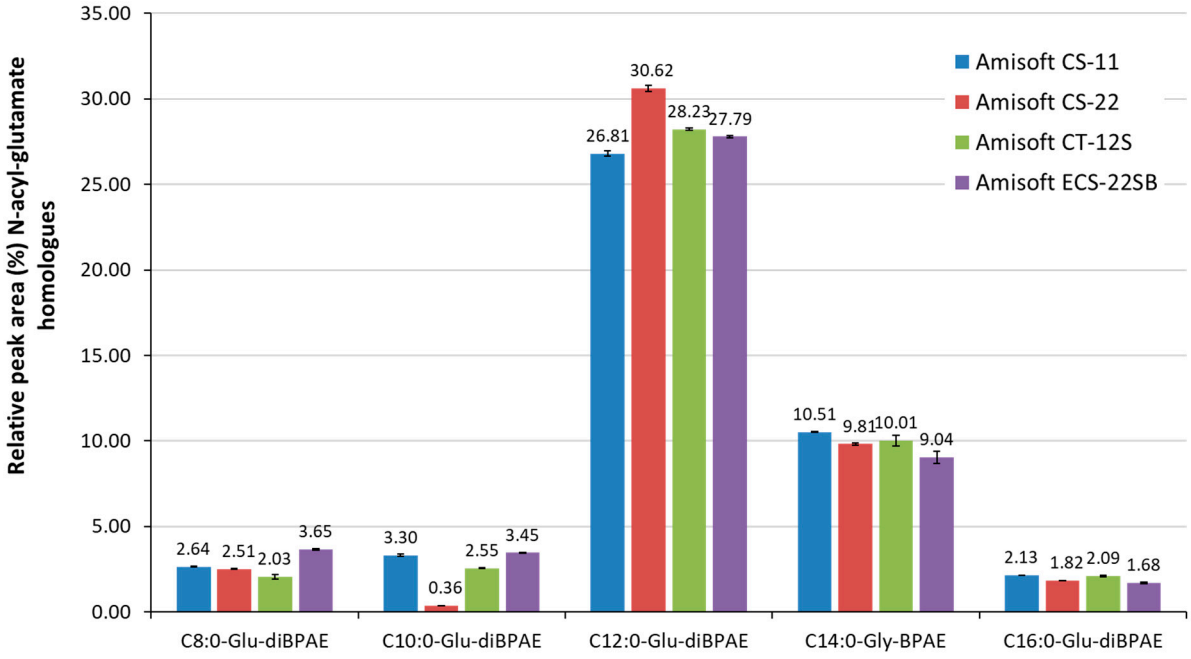


**Figure S2.** Relative content of *N*-acyl-glutamate homologues (expressed as relative peak area of a homologue with respect to surfactants in the chromatogram) determined using LC after derivatization with 2,4'-dibromoacetophenone, yielding corresponding di-4'-bromophenacyl esters (diBPAE). The data are shown as average value of three repetitive analyses with error bars as the standard deviations.

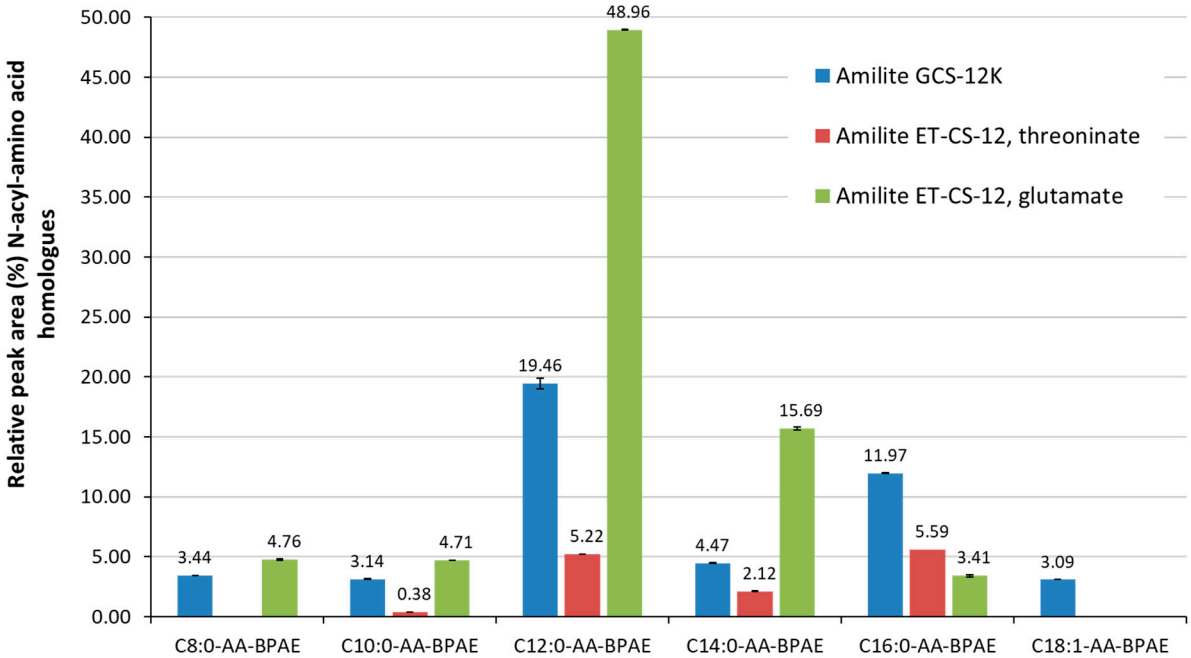


**Figure S3.** Relative content of *N*-acyl-amino acid (AA) homologues (expressed as relative peak area of a homologue with respect to surfactants in the chromatogram) determined using LC after derivatization with 2,4'-dibromoacetophenone, yielding corresponding mono- (BPAE) and di-4'-bromophenacyl esters (diBPAE). The data are shown as average value of three repetitive analyses with error bars as the standard deviations.