

Figure S1. Representative chromatograms of analytes in HRMS Orbitrap with PRM mode in MCF-7 medium (indicated as CTRL) and in MCF-7 EM (indicated as Standard): (A) N-Acetyl-putrescine, (B) Putrescine, (C) Agmatine, (D) N(1)-Acetyl-spermidine, (E) Spermidine, (F) N1-Acetylspermine, (G) Spermine.

Table S1. Calibration curves were prepared for each analyte, which were linear, and the calibration regression coefficients ranged from 0.99 to 0.9965 for all analytes:

| ANALYTE | FORMULA | EXPERIMENTAL MASS (<i>m/z</i>) | THEORETICAL MASS (<i>m/z</i>) | FRAGMENT MASS | R ² |
|--------------------|--|-------------------------------------|------------------------------------|---------------|----------------|
| PUTRESCINE | C ₄ H ₁₂ N ₂ | 89.10743 | 89.10732 | 72.08128 | 0.9991 |
| SPERMIDINE | C ₇ H ₁₉ N ₃ | 146.16505 | 146.16517 | 72.08077 | 0.9925 |
| SPERMINE | C ₁₀ H ₂₆ N ₄ | 203.22232 | 203.22302 | 129.13844 | 0.9900 |
| AGMATINE | C ₅ H ₁₄ N ₄ | 131.12906 | 131.12912 | 114.10258 | 0.9965 |
| N-ACETYLPUTRESCINE | C ₆ H ₁₄ N ₂ O | 131.11783 | 131.11789 | 114.09124 | 0.9960 |
| N-ACETYLSPERMINE | C ₁₂ H ₂₈ N ₄ O | 245.23291 | 245.23359 | 100.07568 | 0.9934 |
| N-ACETYLSPERMIDINE | C ₉ H ₂₁ N ₃ O | 188.1756 | 188.17574 | 171.14909 | 0.9947 |

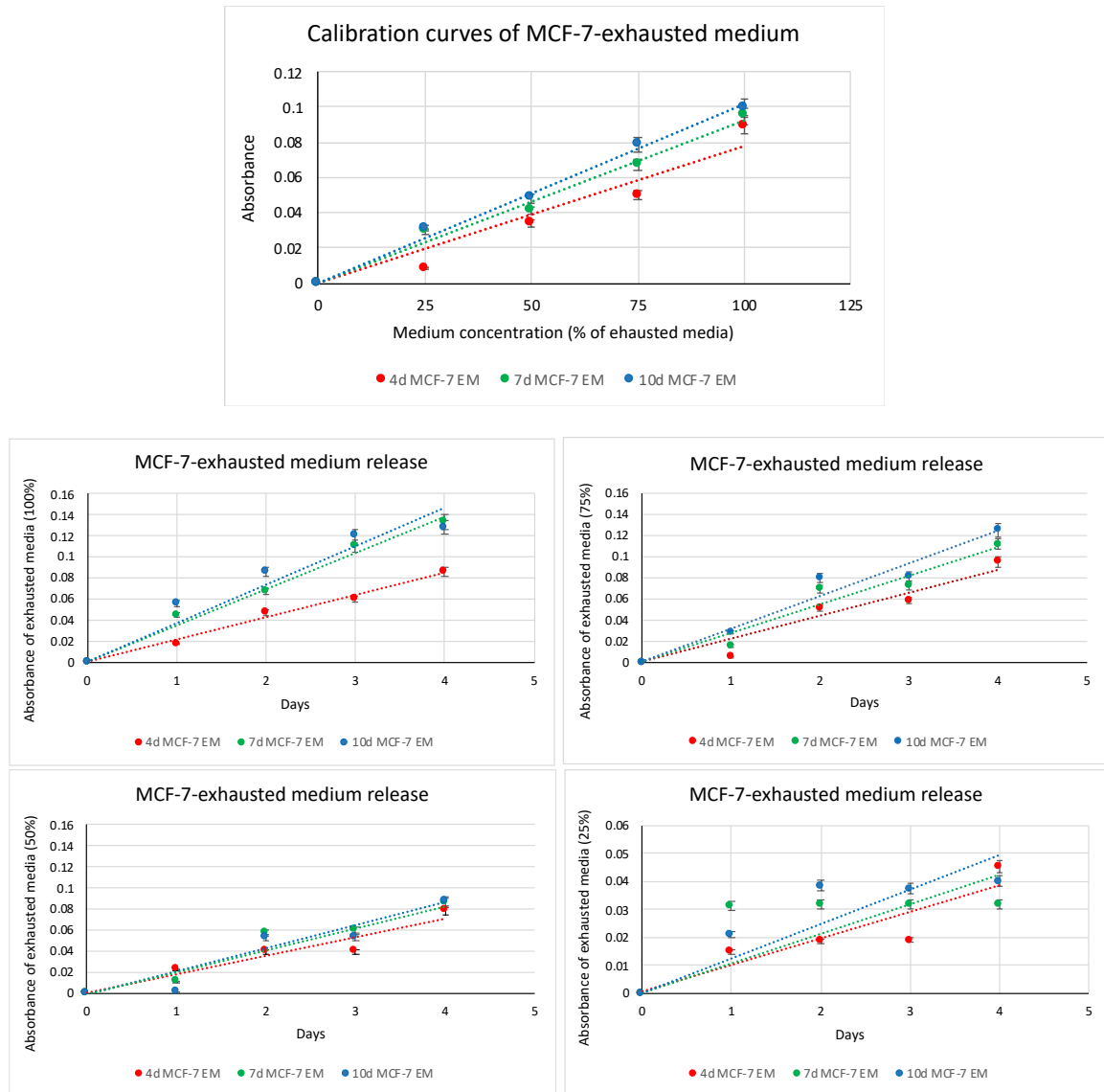


Figure S2. Calibration curve and medium release. MCF-7 cell culture medium was composed of Dulbecco's modified Eagle's Medium (DMEM), (Life Technologies Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS) (Life Technologies, Grand Island, NY, USA), 200 mM L-glutamine (Euroclone, Italy), and 200 U/mL penicillin—0.1 mg/mL streptomycin (Euroclone, Milano, Italy). The exhausted medium was collected from MCF-7, after 4 (4d-MCF-7-EM), 7 (7d-MCF-7-EM), and 10 (10d-MCF-7-EM) days in culture. Calibration curve was prepared by spectrophotometric absorbance readings of scalar dilutions of the MCF-7-exhausted medium (0, 25%, 50%, 75% and 100%). We then monitored over time the variation of absorbance of the various media and observed that only the pure media (100%), was the only one able to guarantee a constant release of its components for each observed time points.