**Supporting Information**

**Comprehensive evaluation of zwitterionic hydrophilic liquid chromatography stationary phases for oligonucleotide characterization**

Honorine Lardeux1,2, Davy Guillarme1,2, Valentina D’Atri1,2,\*

1 School of Pharmaceutical Sciences, University of Geneva, CMU - Rue Michel Servet 1, 1211 Geneva 4, Switzerland 2 Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU - Rue Michel Servet 1, 1211 Geneva 4, Switzerland

Valentina.datri@unige.ch

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**Figure S1.** Structures of the stationary phases for each investigated column, as disclosed by the providers. Gray balls stand for silica particles while red ball stands for polymer beads.



**Figure S2.** Chromatograms of rU15-30 mixture obtained by using the C18 column (a), the amide column (b), the OH5 column (c) and the zwitterionic columns (d – j). For chromatographic conditions and specifications on the columns, please refer to Section 2.4.2 and Table 2, respectively.

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**Table S1.** Tanaka test: Normalized data related to the radar plots reported in Figure 1.For each HILIC column, normalized values (indicated by a N) were obtained by dividing original data reported in Table S2 by *MeanZwit*, the average values of zwitterionic columns.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Column** | **kN(U)** | **αN(CH3)** | **αN(OH)** | **αN(V/A)** | **αN(2d/3d)** | **αN(AX)** | **αN(CX)** | **αN(Tb/Tp)** |
| Amide | 0.93 | 0.93 | 1.02 | 0.88 | 0.97 | 0.47 | 0.47 | 1.30 |
| OH5 | 0.41 | 0.86 | 0.80 | 0.85 | 0.99 | 0.61 | 2.00 | 1.22 |
| Z-HILIC | 1.65 | 1.09 | 1.14 | 1.08 | 1.00 | 1.77 | 0.26 | 1.00 |
| ZIC-cHILIC | 1.37 | 1.06 | 1.14 | 1.14 | 1.02 | 1.18 | 0.17 | 0.94 |
| ZIC-pHILIC | 1.65 | 1.22 | 1.21 | 0.97 | 1.01 | 1.17 | 0.37 | 1.00 |
| Nucleodur HILIC | 0.57 | 0.90 | 0.88 | 0.95 | 0.99 | 0.58 | 1.62 | 1.00 |
| Nucleoshell HILIC | 0.47 | 0.90 | 0.86 | 0.94 | 1.00 | 0.41 | 2.19 | 1.05 |
| HILIC-Z | 0.66 | 0.93 | 0.91 | 0.96 | 0.99 | 1.28 | 0.80 | 1.00 |
| Syncronis HILIC | 0.63 | 0.90 | 0.87 | 0.95 | 0.99 | 0.61 | 1.60 | 1.00 |

**Table S2.** Tanaka test: Average values used to calculate the normalized data reported in Table S1. For each HILIC column, average values were obtained from the raw data reported in Table S3.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Columns** | **k(U)** | **α(CH3)** | **α(OH)** | **α(V/A)** | **α(2d/3d)** | **α(AX)** | **α(CX)** | **α(Tb/Tp)** |
| Amide | 2.37 | 1.29 | 1.70 | 1.29 | 1.07 | 0.14 | 1.02 | 1.30 |
| OH5 | 1.03 | 1.20 | 1.33 | 1.25 | 1.09 | 0.19 | 4.32 | 1.22 |
| Z-HILIC | 4.19 | 1.52 | 1.90 | 1.57 | 1.11 | 0.55 | 0.56 | 1.00 |
| ZIC-cHILIC | 3.49 | 1.47 | 1.90 | 1.67 | 1.12 | 0.37 | 0.37 | 0.93 |
| ZIC-pHILIC | 4.21 | 1.69 | 2.03 | 1.42 | 1.11 | 0.36 | 0.80 | 1.00 |
| Nucleodur HILIC | 1.44 | 1.25 | 1.47 | 1.39 | 1.09 | 0.18 | 3.50 | 1.00 |
| Nucleoshell HILIC | 1.20 | 1.25 | 1.43 | 1.37 | 1.10 | 0.13 | 4.73 | 1.05 |
| HILIC-Z | 1.67 | 1.28 | 1.52 | 1.40 | 1.09 | 0.40 | 1.73 | 1.00 |
| Syncronis HILIC | 1.59 | 1.25 | 1.46 | 1.39 | 1.09 | 0.19 | 3.45 | 1.00 |
| MinValue | 1.03 | 1.20 | 1.33 | 1.25 | 1.07 | 0.13 | 0.37 | 0.93 |
| MaxValue | 4.21 | 1.69 | 2.03 | 1.67 | 1.12 | 0.55 | 4.73 | 1.30 |
| MeanZwit | 2.54 | 1.39 | 1.67 | 1.46 | 1.10 | 0.31 | 2.16 | 1.00 |

**Table S3.** Tanaka test: Raw data. For each HILIC column, 5 replicate injections were performed, and t0 and retention times used to calculate the retention factors and test parameters were reported.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Amide** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) =  kU/ k5MU | t0 | U | 2dU | k U | k 2dU | α(OH) = kU / k2dU | t0 | A | V | k A | k V | α(V/A) = kV / kA |
| 1 | 0.470 | 1.586 | 1.335 | 2.374 | 1.840 | 1.290 | 0.470 | 1.586 | 1.125 | 2.374 | 1.394 | 1.704 | 0.470 | 1.663 | 2.007 | 2.538 | 3.270 | 1.288 |
| 2 | 0.470 | 1.587 | 1.335 | 2.377 | 1.840 | 1.291 | 0.470 | 1.586 | 1.125 | 2.374 | 1.394 | 1.704 | 0.470 | 1.664 | 2.007 | 2.540 | 3.270 | 1.287 |
| 3 | 0.470 | 1.586 | 1.334 | 2.374 | 1.838 | 1.292 | 0.470 | 1.586 | 1.126 | 2.374 | 1.396 | 1.701 | 0.470 | 1.664 | 2.007 | 2.540 | 3.270 | 1.287 |
| 4 | 0.470 | 1.586 | 1.334 | 2.374 | 1.838 | 1.292 | 0.470 | 1.586 | 1.126 | 2.374 | 1.396 | 1.701 | 0.470 | 1.663 | 2.007 | 2.538 | 3.270 | 1.288 |
| 5 | 0.470 | 1.586 | 1.334 | 2.374 | 1.838 | 1.292 | 0.470 | 1.586 | 1.125 | 2.374 | 1.394 | 1.704 | 0.470 | 1.663 | 2.007 | 2.538 | 3.270 | 1.288 |
| Average | 0.470 | 1.586 | 1.334 | 2.375 | 1.839 | 1.291 | 0.470 | 1.586 | 1.125 | 2.374 | 1.394 | 1.703 | 0.470 | 1.663 | 2.007 | 2.539 | 3.270 | 1.288 |
| Std Dev | 0.0E+00 | 4.5E-04 | 5.5E-04 | 9.5E-04 | 1.2E-03 | 6.5E-04 | 0.0E+00 | 0.0E+00 | 5.5E-04 | 0.0E+00 | 1.2E-03 | 1.4E-03 | 0.0E+00 | 5.5E-04 | 0.0E+00 | 1.2E-03 | 0.0E+00 | 5.9E-04 |
| %RSD | 0.00 | 0.03 | 0.04 | 0.04 | 0.06 | 0.05 | 0.00 | 0.00 | 0.05 | 0.00 | 0.08 | 0.08 | 0.00 | 0.03 | 0.00 | 0.05 | 0.00 | 0.05 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Amide** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | α(2dG/3dG) = k2dG / k3dG | t0 | U | SPTS | k U | k SPTS | α(AX) = kSPTS / kU | t0 | U | TMPAC | k U | k TMPAC | α(CX) = kTMPAC / kU |
| 1 | 0.470 | 2.867 | 2.712 | 5.100 | 4.770 | 1.069 | 0.470 | 1.586 | 0.631 | 2.374 | 0.343 | 0.144 | 0.470 | 1.586 | 1.603 | 2.374 | 2.411 | 1.015 |
| 2 | 0.470 | 2.867 | 2.713 | 5.100 | 4.772 | 1.069 | 0.470 | 1.586 | 0.631 | 2.374 | 0.343 | 0.144 | 0.470 | 1.586 | 1.603 | 2.374 | 2.411 | 1.015 |
| 3 | 0.470 | 2.867 | 2.712 | 5.100 | 4.770 | 1.069 | 0.470 | 1.586 | 0.631 | 2.374 | 0.343 | 0.144 | 0.470 | 1.586 | 1.603 | 2.374 | 2.411 | 1.015 |
| 4 | 0.470 | 2.867 | 2.712 | 5.100 | 4.770 | 1.069 | 0.470 | 1.586 | 0.631 | 2.374 | 0.343 | 0.144 | 0.470 | 1.586 | 1.604 | 2.374 | 2.413 | 1.016 |
| 5 | 0.470 | 2.867 | 2.712 | 5.100 | 4.770 | 1.069 | 0.470 | 1.586 | 0.631 | 2.374 | 0.343 | 0.144 | 0.470 | 1.586 | 1.604 | 2.374 | 2.413 | 1.016 |
| Average | 0.470 | 2.867 | 2.712 | 5.100 | 4.771 | 1.069 | 0.470 | 1.586 | 0.631 | 2.374 | 0.343 | 0.144 | 0.470 | 1.586 | 1.603 | 2.374 | 2.411 | 1.016 |
| Std Dev | 0.0E+00 | 0.0E+00 | 4.5E-04 | 0.0E+00 | 9.5E-04 | 2.1E-04 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 5.5E-04 | 0.0E+00 | 1.2E-03 | 4.9E-04 |
| %RSD | 0.00 | 0.00 | 0.02 | 0.00 | 0.02 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.03 | 0.00 | 0.05 | 0.05 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Amide** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | α(Tb/Tp) = kTb / kTp |
| 1 | 0.470 | 0.779 | 0.707 | 0.657 | 0.504 | 1.304 |
| 2 | 0.470 | 0.779 | 0.707 | 0.657 | 0.504 | 1.304 |
| 3 | 0.470 | 0.779 | 0.708 | 0.657 | 0.506 | 1.298 |
| 4 | 0.470 | 0.779 | 0.707 | 0.657 | 0.504 | 1.304 |
| 5 | 0.470 | 0.779 | 0.708 | 0.657 | 0.506 | 1.298 |
| Average | 0.470 | 0.779 | 0.707 | 0.657 | 0.505 | 1.302 |
| Std Dev | 0.0E+00 | 0.0E+00 | 5.5E-04 | 0.0E+00 | 1.2E-03 | 3.0E-03 |
| %RSD | 0.00 | 0.00 | 0.08 | 0.00 | 0.23 | 0.23 |
|  |  |  |  |  |  |  |
| **OH5** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) =  kU/ k5MU | t0 | U | 2dU | k U | k 2dU | α(OH) = kU / k2dU | t0 | A | V | k A | k V | α(V/A) = kV / kA |
| 1 | 0.451 | 0.917 | 0.841 | 1.033 | 0.865 | 1.195 | 0.451 | 0.917 | 0.801 | 1.033 | 0.776 | 1.331 | 0.451 | 1.271 | 1.473 | 1.818 | 2.266 | 1.246 |
| 2 | 0.452 | 0.918 | 0.841 | 1.031 | 0.861 | 1.198 | 0.451 | 0.918 | 0.801 | 1.035 | 0.776 | 1.334 | 0.452 | 1.271 | 1.473 | 1.812 | 2.259 | 1.247 |
| 3 | 0.451 | 0.917 | 0.840 | 1.033 | 0.863 | 1.198 | 0.452 | 0.918 | 0.801 | 1.031 | 0.772 | 1.335 | 0.451 | 1.271 | 1.472 | 1.818 | 2.264 | 1.245 |
| 4 | 0.451 | 0.917 | 0.840 | 1.033 | 0.863 | 1.198 | 0.452 | 0.918 | 0.802 | 1.031 | 0.774 | 1.331 | 0.451 | 1.270 | 1.472 | 1.816 | 2.264 | 1.247 |
| 5 | 0.451 | 0.917 | 0.840 | 1.033 | 0.863 | 1.198 | 0.452 | 0.918 | 0.802 | 1.031 | 0.774 | 1.331 | 0.451 | 1.270 | 1.472 | 1.816 | 2.264 | 1.247 |
| Average | 0.451 | 0.917 | 0.840 | 1.033 | 0.863 | 1.197 | 0.452 | 0.918 | 0.801 | 1.032 | 0.775 | 1.333 | 0.451 | 1.271 | 1.472 | 1.816 | 2.263 | 1.246 |
| Std Dev | 4.5E-04 | 4.5E-04 | 5.5E-04 | 1.0E-03 | 1.5E-03 | 1.4E-03 | 5.5E-04 | 4.5E-04 | 5.5E-04 | 2.0E-03 | 1.6E-03 | 1.9E-03 | 4.5E-04 | 5.5E-04 | 5.5E-04 | 2.5E-03 | 2.7E-03 | 6.6E-04 |
| %RSD | 0.10 | 0.05 | 0.07 | 0.10 | 0.17 | 0.11 | 0.12 | 0.05 | 0.07 | 0.20 | 0.21 | 0.14 | 0.10 | 0.04 | 0.04 | 0.14 | 0.12 | 0.05 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **OH5** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | α(2dG/3dG) = k2dG / k3dG | t0 | U | SPTS | k U | k SPTS | α(AX) = kSPTS / kU | t0 | U | TMPAC | k U | k TMPAC | α(CX) = kTMPAC / kU |
| 1 | 0.451 | 1.794 | 1.678 | 2.978 | 2.721 | 1.095 | 0.451 | 0.917 | 0.539 | 1.033 | 0.195 | 0.189 | 0.451 | 0.917 | 2.466 | 1.033 | 4.468 | 4.324 |
| 2 | 0.452 | 1.795 | 1.679 | 2.971 | 2.715 | 1.095 | 0.451 | 0.918 | 0.540 | 1.035 | 0.197 | 0.191 | 0.451 | 0.918 | 2.466 | 1.035 | 4.468 | 4.315 |
| 3 | 0.451 | 1.795 | 1.678 | 2.980 | 2.721 | 1.095 | 0.451 | 0.918 | 0.539 | 1.035 | 0.195 | 0.188 | 0.451 | 0.918 | 2.466 | 1.035 | 4.468 | 4.315 |
| 4 | 0.451 | 1.794 | 1.678 | 2.978 | 2.721 | 1.095 | 0.451 | 0.918 | 0.540 | 1.035 | 0.197 | 0.191 | 0.451 | 0.918 | 2.466 | 1.035 | 4.468 | 4.315 |
| 5 | 0.451 | 1.794 | 1.678 | 2.978 | 2.721 | 1.095 | 0.451 | 0.918 | 0.539 | 1.035 | 0.195 | 0.188 | 0.451 | 0.918 | 2.466 | 1.035 | 4.468 | 4.315 |
| Average | 0.451 | 1.794 | 1.678 | 2.977 | 2.719 | 1.095 | 0.451 | 0.918 | 0.539 | 1.035 | 0.196 | 0.189 | 0.451 | 0.918 | 2.466 | 1.035 | 4.468 | 4.317 |
| Std Dev | 4.5E-04 | 5.5E-04 | 4.5E-04 | 3.3E-03 | 2.7E-03 | 3.6E-04 | 0.0E+00 | 4.5E-04 | 5.5E-04 | 9.9E-04 | 1.2E-03 | 1.1E-03 | 0.0E+00 | 4.5E-04 | 0.0E+00 | 9.9E-04 | 0.0E+00 | 4.1E-03 |
| %RSD | 0.10 | 0.03 | 0.03 | 0.11 | 0.10 | 0.03 | 0.00 | 0.05 | 0.10 | 0.10 | 0.62 | 0.59 | 0.00 | 0.05 | 0.00 | 0.10 | 0.00 | 0.10 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **OH5** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | α(Tb/Tp) = kTb / kTp |
| 1 | 0.451 | 0.686 | 0.644 | 0.521 | 0.428 | 1.218 |
| 2 | 0.451 | 0.686 | 0.644 | 0.521 | 0.428 | 1.218 |
| 3 | 0.451 | 0.687 | 0.644 | 0.523 | 0.428 | 1.223 |
| 4 | 0.451 | 0.687 | 0.644 | 0.523 | 0.428 | 1.223 |
| 5 | 0.451 | 0.686 | 0.644 | 0.521 | 0.428 | 1.218 |
| Average | 0.451 | 0.686 | 0.644 | 0.522 | 0.428 | 1.220 |
| Std Dev | 0.0E+00 | 5.5E-04 | 0.0E+00 | 1.2E-03 | 0.0E+00 | 2.8E-03 |
| %RSD | 0.00 | 0.08 | 0.00 | 0.23 | 0.00 | 0.23 |

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Z-HILIC** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) =  kU/ k5MU | t0 | U | 2dU | k U | k 2dU | α(OH) =  kU / k2dU | t0 | A | V | k A | k V | α(V/A) = kV / kA |
| 1 | 0.370 | 1.927 | 1.396 | 4.208 | 2.773 | 1.518 | 0.371 | 1.927 | 1.190 | 4.194 | 2.208 | 1.900 | 0.371 | 1.742 | 2.529 | 3.695 | 5.817 | 1.574 |
| 2 | 0.371 | 1.927 | 1.397 | 4.194 | 2.765 | 1.517 | 0.371 | 1.927 | 1.190 | 4.194 | 2.208 | 1.900 | 0.371 | 1.742 | 2.529 | 3.695 | 5.817 | 1.574 |
| 3 | 0.371 | 1.927 | 1.397 | 4.194 | 2.765 | 1.517 | 0.371 | 1.927 | 1.190 | 4.194 | 2.208 | 1.900 | 0.371 | 1.742 | 2.529 | 3.695 | 5.817 | 1.574 |
| 4 | 0.371 | 1.927 | 1.397 | 4.194 | 2.765 | 1.517 | 0.371 | 1.927 | 1.189 | 4.194 | 2.205 | 1.902 | 0.371 | 1.742 | 2.529 | 3.695 | 5.817 | 1.574 |
| 5 | 0.371 | 1.927 | 1.397 | 4.194 | 2.765 | 1.517 | 0.371 | 1.927 | 1.190 | 4.194 | 2.208 | 1.900 | 0.371 | 1.742 | 2.529 | 3.695 | 5.817 | 1.574 |
| Average | 0.371 | 1.927 | 1.397 | 4.197 | 2.767 | 1.517 | 0.371 | 1.927 | 1.190 | 4.194 | 2.207 | 1.900 | 0.371 | 1.742 | 2.529 | 3.695 | 5.817 | 1.574 |
| Std Dev | 4.5E-04 | 0.0E+00 | 4.5E-04 | 6.3E-03 | 3.3E-03 | 4.4E-04 | 0.0E+00 | 0.0E+00 | 4.5E-04 | 0.0E+00 | 1.2E-03 | 1.0E-03 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 0.0E+00 | 0.0E+00 |
| %RSD | 0.12 | 0.00 | 0.03 | 0.15 | 0.12 | 0.03 | 0.00 | 0.00 | 0.04 | 0.00 | 0.05 | 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Z-HILIC** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | α(2dG/3dG) = k2dG / k3dG | t0 | U | SPTS | k U | k SPTS | α(AX) = kSPTS / kU | t0 | U | TMPAC | k U | k TMPAC | α(CX) = kTMPAC / kU |
| 1 | 0.371 | 4.386 | 4.004 | 10.822 | 9.792 | 1.105 | 0.371 | 1.927 | 1.225 | 4.194 | 2.302 | 0.549 | 0.371 | 1.927 | 1.24 | 4.194 | 2.342 | 0.558 |
| 2 | 0.371 | 4.386 | 4.004 | 10.822 | 9.792 | 1.105 | 0.371 | 1.927 | 1.224 | 4.194 | 2.299 | 0.548 | 0.371 | 1.927 | 1.24 | 4.194 | 2.342 | 0.558 |
| 3 | 0.370 | 4.386 | 4.004 | 10.854 | 9.822 | 1.105 | 0.371 | 1.927 | 1.225 | 4.194 | 2.302 | 0.549 | 0.371 | 1.927 | 1.24 | 4.194 | 2.342 | 0.558 |
| 4 | 0.370 | 4.385 | 4.004 | 10.851 | 9.822 | 1.105 | 0.371 | 1.927 | 1.225 | 4.194 | 2.302 | 0.549 | 0.371 | 1.927 | 1.239 | 4.194 | 2.340 | 0.558 |
| 5 | 0.371 | 4.386 | 4.005 | 10.822 | 9.795 | 1.105 | 0.371 | 1.927 | 1.224 | 4.194 | 2.299 | 0.548 | 0.370 | 1.927 | 1.239 | 4.208 | 2.349 | 0.558 |
| Average | 0.371 | 4.386 | 4.004 | 10.834 | 9.805 | 1.105 | 0.371 | 1.927 | 1.225 | 4.194 | 2.301 | 0.549 | 0.371 | 1.927 | 1.240 | 4.197 | 2.343 | 0.558 |
| Std Dev | 5.5E-04 | 4.5E-04 | 4.5E-04 | 1.7E-02 | 1.6E-02 | 1.6E-04 | 0.0E+00 | 0.0E+00 | 5.5E-04 | 0.0E+00 | 1.5E-03 | 3.5E-04 | 4.5E-04 | 0.0E+00 | 5.5E-04 | 6.3E-03 | 3.3E-03 | 2.9E-04 |
| %RSD | 0.15 | 0.01 | 0.01 | 0.15 | 0.16 | 0.01 | 0.00 | 0.00 | 0.04 | 0.00 | 0.06 | 0.06 | 0.12 | 0.00 | 0.04 | 0.15 | 0.14 | 0.05 |
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| **Z-HILIC** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | α(Tb/Tp) = kTb / kTp |
| 1 | 0.371 | 0.591 | 0.591 | 0.593 | 0.593 | 1.000 |
| 2 | 0.371 | 0.591 | 0.591 | 0.593 | 0.593 | 1.000 |
| 3 | 0.371 | 0.591 | 0.591 | 0.593 | 0.593 | 1.000 |
| 4 | 0.371 | 0.591 | 0.591 | 0.593 | 0.593 | 1.000 |
| 5 | 0.371 | 0.592 | 0.592 | 0.596 | 0.596 | 1.000 |
| Average | 0.371 | 0.591 | 0.591 | 0.594 | 0.594 | 1.000 |
| Std Dev | 0.0E+00 | 4.5E-04 | 4.5E-04 | 1.2E-03 | 1.2E-03 | 0.0E+00 |
| %RSD | 0.00 | 0.08 | 0.08 | 0.20 | 0.20 | 0.00 |

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **ZIC-cHILIC** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) =  kU/ k5MU | t0 | U | 2dU | k U | k 2dU | α(OH) = kU / k2dU | t0 | A | V | k A | k V | α(V/A) = kV / kA |
| 1 | 0.629 | 2.831 | 2.129 | 3.501 | 2.385 | 1.468 | 0.630 | 2.828 | 1.786 | 3.489 | 1.835 | 1.901 | 0.630 | 2.353 | 3.506 | 2.735 | 4.565 | 1.669 |
| 2 | 0.629 | 2.830 | 2.128 | 3.499 | 2.383 | 1.468 | 0.630 | 2.828 | 1.786 | 3.489 | 1.835 | 1.901 | 0.630 | 2.352 | 3.505 | 2.733 | 4.563 | 1.670 |
| 3 | 0.629 | 2.829 | 2.127 | 3.498 | 2.382 | 1.469 | 0.630 | 2.828 | 1.786 | 3.489 | 1.835 | 1.901 | 0.630 | 2.353 | 3.506 | 2.735 | 4.565 | 1.669 |
| 4 | 0.629 | 2.828 | 2.127 | 3.496 | 2.382 | 1.468 | 0.630 | 2.827 | 1.785 | 3.487 | 1.833 | 1.902 | 0.630 | 2.352 | 3.505 | 2.733 | 4.563 | 1.670 |
| 5 | 0.629 | 2.828 | 2.126 | 3.496 | 2.380 | 1.469 | 0.630 | 2.827 | 1.785 | 3.487 | 1.833 | 1.902 | 0.630 | 2.352 | 3.505 | 2.733 | 4.563 | 1.670 |
| Average | 0.629 | 2.829 | 2.127 | 3.498 | 2.382 | 1.468 | 0.630 | 2.828 | 1.786 | 3.488 | 1.834 | 1.902 | 0.630 | 2.352 | 3.505 | 2.734 | 4.564 | 1.669 |
| Std Dev | 0.0E+00 | 1.3E-03 | 1.1E-03 | 2.1E-03 | 1.8E-03 | 4.2E-04 | 0.0E+00 | 5.5E-04 | 5.5E-04 | 8.7E-04 | 8.7E-04 | 4.3E-04 | 0.0E+00 | 5.5E-04 | 5.5E-04 | 8.7E-04 | 8.7E-04 | 2.1E-04 |
| %RSD | 0.00 | 0.05 | 0.05 | 0.06 | 0.08 | 0.03 | 0.00 | 0.02 | 0.03 | 0.02 | 0.05 | 0.02 | 0.00 | 0.02 | 0.02 | 0.03 | 0.02 | 0.01 |
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| **ZIC-cHILIC** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | α(2dG/3dG) = k2dG / k3dG | t0 | U | SPTS | k U | k SPTS | α(AX) = kSPTS / kU | t0 | U | TMPAC | k U | k TMPAC | α(CX) = kTMPAC / kU |
| 1 | 0.630 | 5.945 | 5.378 | 8.437 | 7.537 | 1.119 | 0.630 | 2.828 | 1.437 | 3.489 | 1.281 | 0.367 | 0.631 | 2.828 | 1.438 | 3.482 | 1.279 | 0.367 |
| 2 | 0.630 | 5.944 | 5.377 | 8.435 | 7.535 | 1.119 | 0.630 | 2.828 | 1.438 | 3.489 | 1.283 | 0.368 | 0.631 | 2.828 | 1.438 | 3.482 | 1.279 | 0.367 |
| 3 | 0.630 | 5.944 | 5.377 | 8.435 | 7.535 | 1.119 | 0.630 | 2.828 | 1.438 | 3.489 | 1.283 | 0.368 | 0.630 | 2.828 | 1.437 | 3.489 | 1.281 | 0.367 |
| 4 | 0.631 | 5.945 | 5.377 | 8.422 | 7.521 | 1.120 | 0.630 | 2.827 | 1.437 | 3.487 | 1.281 | 0.367 | 0.631 | 2.827 | 1.438 | 3.480 | 1.279 | 0.367 |
| 5 | 0.630 | 5.943 | 5.375 | 8.433 | 7.532 | 1.120 | 0.630 | 2.827 | 1.437 | 3.487 | 1.281 | 0.367 | 0.630 | 2.827 | 1.437 | 3.487 | 1.281 | 0.367 |
| Average | 0.630 | 5.944 | 5.377 | 8.432 | 7.532 | 1.120 | 0.630 | 2.828 | 1.437 | 3.488 | 1.282 | 0.367 | 0.631 | 2.828 | 1.438 | 3.484 | 1.280 | 0.367 |
| Std Dev | 4.5E-04 | 8.4E-04 | 1.1E-03 | 6.1E-03 | 6.1E-03 | 1.4E-04 | 0.0E+00 | 5.5E-04 | 5.5E-04 | 8.7E-04 | 8.7E-04 | 2.0E-04 | 5.5E-04 | 5.5E-04 | 5.5E-04 | 3.8E-03 | 1.1E-03 | 1.2E-04 |
| %RSD | 0.07 | 0.01 | 0.02 | 0.07 | 0.08 | 0.01 | 0.00 | 0.02 | 0.04 | 0.02 | 0.07 | 0.05 | 0.09 | 0.02 | 0.04 | 0.11 | 0.09 | 0.03 |
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| **ZIC-cHILIC** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | α(Tb/Tp) = kTb / kTp |
| 1 | 0.631 | 0.925 | 0.946 | 0.466 | 0.499 | 0.933 |
| 2 | 0.631 | 0.926 | 0.946 | 0.468 | 0.499 | 0.937 |
| 3 | 0.631 | 0.924 | 0.946 | 0.464 | 0.499 | 0.930 |
| 4 | 0.631 | 0.925 | 0.946 | 0.466 | 0.499 | 0.933 |
| 5 | 0.631 | 0.925 | 0.946 | 0.466 | 0.499 | 0.933 |
| Average | 0.631 | 0.925 | 0.946 | 0.466 | 0.499 | 0.933 |
| Std Dev | 0.0E+00 | 7.1E-04 | 0.0E+00 | 1.1E-03 | 0.0E+00 | 2.2E-03 |
| %RSD | 0.00 | 0.08 | 0.00 | 0.24 | 0.00 | 0.24 |

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| **ZIC-pHILIC** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) =  kU/ k5MU | t0 | U | 2dU | k U | k 2dU | α(OH) = kU / k2dU | t0 | A | V | k A | k V | α(V/A) = kV / kA |
| 1 | 0.571 | 2.973 | 1.99 | 4.207 | 2.485 | 1.693 | 0.571 | 2.973 | 1.753 | 4.207 | 2.070 | 2.032 | 0.571 | 2.521 | 3.339 | 3.415 | 4.848 | 1.419 |
| 2 | 0.571 | 2.973 | 1.99 | 4.207 | 2.485 | 1.693 | 0.571 | 2.973 | 1.753 | 4.207 | 2.070 | 2.032 | 0.571 | 2.520 | 3.337 | 3.413 | 4.844 | 1.419 |
| 3 | 0.572 | 2.974 | 1.991 | 4.199 | 2.481 | 1.693 | 0.571 | 2.973 | 1.753 | 4.207 | 2.070 | 2.032 | 0.571 | 2.520 | 3.340 | 3.413 | 4.849 | 1.421 |
| 4 | 0.571 | 2.974 | 1.991 | 4.208 | 2.487 | 1.692 | 0.571 | 2.972 | 1.752 | 4.205 | 2.068 | 2.033 | 0.571 | 2.520 | 3.338 | 3.413 | 4.846 | 1.420 |
| 5 | 0.572 | 2.974 | 1.991 | 4.199 | 2.481 | 1.693 | 0.571 | 2.972 | 1.752 | 4.205 | 2.068 | 2.033 | 0.571 | 2.520 | 3.338 | 3.413 | 4.846 | 1.420 |
| Average | 0.571 | 2.974 | 1.991 | 4.204 | 2.484 | 1.693 | 0.571 | 2.973 | 1.753 | 4.206 | 2.069 | 2.032 | 0.571 | 2.520 | 3.338 | 3.414 | 4.847 | 1.420 |
| Std Dev | 5.5E-04 | 5.5E-04 | 5.5E-04 | 4.4E-03 | 2.8E-03 | 2.2E-04 | 0.0E+00 | 5.5E-04 | 5.5E-04 | 9.6E-04 | 9.6E-04 | 4.8E-04 | 0.0E+00 | 4.5E-04 | 1.1E-03 | 7.8E-04 | 2.0E-03 | 5.8E-04 |
| %RSD | 0.10 | 0.02 | 0.03 | 0.10 | 0.11 | 0.01 | 0.00 | 0.02 | 0.03 | 0.02 | 0.05 | 0.02 | 0.00 | 0.02 | 0.03 | 0.02 | 0.04 | 0.04 |
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| **ZIC-pHILIC** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | α(2dG/3dG) = k2dG / k3dG | t0 | U | SPTS | k U | k SPTS | α(AX) = kSPTS / kU | t0 | U | TMPAC | k U | k TMPAC | α(CX) = kTMPAC / kU |
| 1 | 0.570 | 6.135 | 5.567 | 9.763 | 8.767 | 1.114 | 0.571 | 2.973 | 1.442 | 4.207 | 1.525 | 0.363 | 0.571 | 2.973 | 2.484 | 4.207 | 3.350 | 0.796 |
| 2 | 0.571 | 6.134 | 5.568 | 9.743 | 8.751 | 1.113 | 0.571 | 2.973 | 1.440 | 4.207 | 1.522 | 0.362 | 0.572 | 2.973 | 2.487 | 4.198 | 3.348 | 0.798 |
| 3 | 0.571 | 6.133 | 5.566 | 9.741 | 8.748 | 1.114 | 0.571 | 2.973 | 1.441 | 4.207 | 1.524 | 0.362 | 0.571 | 2.973 | 2.489 | 4.207 | 3.359 | 0.799 |
| 4 | 0.571 | 6.134 | 5.569 | 9.743 | 8.753 | 1.113 | 0.571 | 2.972 | 1.441 | 4.205 | 1.524 | 0.362 | 0.571 | 2.972 | 2.485 | 4.205 | 3.352 | 0.797 |
| 5 | 0.571 | 6.134 | 5.570 | 9.743 | 8.755 | 1.113 | 0.571 | 2.972 | 1.442 | 4.205 | 1.525 | 0.363 | 0.571 | 2.972 | 2.48 | 4.205 | 3.343 | 0.795 |
| Average | 0.571 | 6.134 | 5.568 | 9.746 | 8.755 | 1.113 | 0.571 | 2.973 | 1.441 | 4.206 | 1.524 | 0.362 | 0.571 | 2.973 | 2.485 | 4.204 | 3.350 | 0.797 |
| Std Dev | 4.5E-04 | 7.1E-04 | 1.6E-03 | 9.4E-03 | 7.2E-03 | 3.4E-04 | 0.0E+00 | 5.5E-04 | 8.4E-04 | 9.6E-04 | 1.5E-03 | 3.8E-04 | 4.5E-04 | 5.5E-04 | 3.4E-03 | 3.8E-03 | 5.8E-03 | 1.3E-03 |
| %RSD | 0.08 | 0.01 | 0.03 | 0.10 | 0.08 | 0.03 | 0.00 | 0.02 | 0.06 | 0.02 | 0.10 | 0.11 | 0.08 | 0.02 | 0.14 | 0.09 | 0.17 | 0.16 |
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| **ZIC-pHILIC** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | α(Tb/Tp) = kTb / kTp |
| 1 | 0.571 | 0.947 | 0.947 | 0.658 | 0.658 | 1.000 |
| 2 | 0.571 | 0.947 | 0.947 | 0.658 | 0.658 | 1.000 |
| 3 | 0.571 | 0.946 | 0.946 | 0.657 | 0.657 | 1.000 |
| 4 | 0.571 | 0.946 | 0.946 | 0.657 | 0.657 | 1.000 |
| 5 | 0.571 | 0.947 | 0.947 | 0.658 | 0.658 | 1.000 |
| Average | 0.571 | 0.947 | 0.947 | 0.658 | 0.658 | 1.000 |
| Std Dev | 0.0E+00 | 5.5E-04 | 5.5E-04 | 9.6E-04 | 9.6E-04 | 0.0E+00 |
| %RSD | 0.00 | 0.06 | 0.06 | 0.15 | 0.15 | 0.00 |

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| **Nucleodur HILIC** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) = kU/ k5MU | t0 | U | 2dU | k U | k 2dU | α(OH) = kU / k2dU | t0 | A | V | k A | k V | | α(V/A) = kV / kA |
| 1 | 0.237 | 0.581 | 0.512 | 1.451 | 1.160 | 1.251 | 0.237 | 0.579 | 0.47 | 1.443 | 0.983 | 1.468 | 0.237 | 0.641 | 0.798 | 1.705 | 2.367 | | 1.389 |
| 2 | 0.237 | 0.581 | 0.512 | 1.451 | 1.160 | 1.251 | 0.237 | 0.579 | 0.47 | 1.443 | 0.983 | 1.468 | 0.237 | 0.641 | 0.798 | 1.705 | 2.367 | | 1.389 |
| 3 | 0.237 | 0.58 | 0.511 | 1.447 | 1.156 | 1.252 | 0.236 | 0.578 | 0.469 | 1.449 | 0.987 | 1.468 | 0.237 | 0.641 | 0.798 | 1.705 | 2.367 | | 1.389 |
| 4 | 0.237 | 0.58 | 0.511 | 1.447 | 1.156 | 1.252 | 0.237 | 0.578 | 0.469 | 1.439 | 0.979 | 1.470 | 0.237 | 0.640 | 0.797 | 1.700 | 2.363 | | 1.390 |
| 5 | 0.237 | 0.579 | 0.511 | 1.443 | 1.156 | 1.248 | 0.237 | 0.578 | 0.470 | 1.439 | 0.983 | 1.464 | 0.237 | 0.641 | 0.797 | 1.705 | 2.363 | | 1.386 |
| Average | 0.237 | 0.580 | 0.511 | 1.448 | 1.158 | 1.251 | 0.237 | 0.578 | 0.470 | 1.443 | 0.983 | 1.467 | 0.237 | 0.641 | 0.798 | 1.704 | 2.365 | | 1.388 |
| Std Dev | 0.0E+00 | 8.4E-04 | 5.5E-04 | 3.5E-03 | 2.3E-03 | 1.5E-03 | 4.5E-04 | 5.5E-04 | 5.5E-04 | 4.2E-03 | 3.0E-03 | 2.3E-03 | 3.1E-17 | 4.5E-04 | 5.5E-04 | 1.9E-03 | 2.3E-03 | | 1.3E-03 |
| %RSD | 0.00 | 0.14 | 0.11 | 0.24 | 0.20 | 0.12 | 0.19 | 0.09 | 0.12 | 0.29 | 0.30 | 0.16 | 0.00 | 0.07 | 0.07 | 0.11 | 0.10 | | 0.09 |
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| **Nucleodur HILIC** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | α(2dG/3dG) = k2dG / k3dG | t0 | U | SPTS | k U | k SPTS | α(AX) = kSPTS / kU | t0 | U | TMPAC | k U | | k TMPAC | α(CX) = kTMPAC / kU |
| 1 | 0.237 | 1.188 | 1.109 | 4.013 | 3.679 | 1.091 | 0.237 | 0.579 | 0.298 | 1.443 | 0.257 | 0.178 | 0.237 | 0.579 | 1.429 | 1.443 | | 5.030 | 3.485 |
| 2 | 0.237 | 1.188 | 1.109 | 4.013 | 3.679 | 1.091 | 0.237 | 0.579 | 0.298 | 1.443 | 0.257 | 0.178 | 0.237 | 0.579 | 1.430 | 1.443 | | 5.034 | 3.488 |
| 3 | 0.237 | 1.188 | 1.108 | 4.013 | 3.675 | 1.092 | 0.237 | 0.578 | 0.299 | 1.439 | 0.262 | 0.182 | 0.237 | 0.578 | 1.431 | 1.439 | | 5.038 | 3.501 |
| 4 | 0.237 | 1.188 | 1.109 | 4.013 | 3.679 | 1.091 | 0.237 | 0.578 | 0.299 | 1.439 | 0.262 | 0.182 | 0.237 | 0.578 | 1.431 | 1.439 | | 5.038 | 3.501 |
| 5 | 0.237 | 1.188 | 1.109 | 4.013 | 3.679 | 1.091 | 0.237 | 0.578 | 0.299 | 1.439 | 0.262 | 0.182 | 0.238 | 0.578 | 1.433 | 1.429 | | 5.021 | 3.515 |
| Average | 0.237 | 1.188 | 1.109 | 4.013 | 3.678 | 1.091 | 0.237 | 0.578 | 0.299 | 1.441 | 0.260 | 0.180 | 0.237 | 0.578 | 1.431 | 1.438 | | 5.032 | 3.498 |
| Std Dev | 3.1E-17 | 0.0E+00 | 4.5E-04 | 0.0E+00 | 1.9E-03 | 5.6E-04 | 0.0E+00 | 5.5E-04 | 5.5E-04 | 2.3E-03 | 2.3E-03 | 1.9E-03 | 4.5E-04 | 5.5E-04 | 1.5E-03 | 5.9E-03 | | 7.1E-03 | 1.2E-02 |
| %RSD | 0.00 | 0.00 | 0.04 | 0.00 | 0.05 | 0.05 | 0.00 | 0.09 | 0.18 | 0.16 | 0.89 | 1.05 | 0.19 | 0.09 | 0.10 | 0.41 | | 0.14 | 0.34 |
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| **Nucleodur HILIC** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | α(Tb/Tp) = kTb / kTp |
| 1 | 0.237 | 0.338 | 0.338 | 0.426 | 0.426 | 1.000 |
| 2 | 0.237 | 0.337 | 0.337 | 0.422 | 0.422 | 1.000 |
| 3 | 0.237 | 0.337 | 0.337 | 0.422 | 0.422 | 1.000 |
| 4 | 0.237 | 0.337 | 0.337 | 0.422 | 0.422 | 1.000 |
| 5 | 0.236 | 0.336 | 0.336 | 0.424 | 0.424 | 1.000 |
| Average | 0.237 | 0.337 | 0.337 | 0.423 | 0.423 | 1.000 |
| Std Dev | 4.5E-04 | 7.1E-04 | 7.1E-04 | 1.9E-03 | 1.9E-03 | 0.0E+00 |
| %RSD | 0.19 | 0.21 | 0.21 | 0.44 | 0.44 | 0.00 |

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| **Nucleoshell**  **HILIC** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) = kU/ k5MU | t0 | U | 2dU | k U | k 2dU | α(OH) = kU / k2dU | t0 | A | V | k A | k V | α(V/A) =  kV / kA |
| 1 | 0.813 | 1.797 | 1.602 | 1.210 | 0.970 | 1.247 | 0.813 | 1.792 | 1.497 | 1.204 | 0.841 | 1.431 | 0.813 | 2.09 | 2.559 | 1.571 | 2.148 | 1.367 |
| 2 | 0.813 | 1.797 | 1.601 | 1.210 | 0.969 | 1.249 | 0.814 | 1.792 | 1.497 | 1.201 | 0.839 | 1.432 | 0.814 | 2.09 | 2.559 | 1.568 | 2.144 | 1.368 |
| 3 | 0.813 | 1.795 | 1.600 | 1.208 | 0.968 | 1.248 | 0.813 | 1.791 | 1.497 | 1.203 | 0.841 | 1.430 | 0.814 | 2.09 | 2.559 | 1.568 | 2.144 | 1.368 |
| 4 | 0.813 | 1.794 | 1.599 | 1.207 | 0.967 | 1.248 | 0.813 | 1.791 | 1.496 | 1.203 | 0.840 | 1.432 | 0.813 | 2.089 | 2.558 | 1.569 | 2.146 | 1.368 |
| 5 | 0.813 | 1.793 | 1.598 | 1.205 | 0.966 | 1.248 | 0.813 | 1.791 | 1.496 | 1.203 | 0.840 | 1.432 | 0.814 | 2.090 | 2.559 | 1.568 | 2.144 | 1.368 |
| Average | 0.813 | 1.795 | 1.600 | 1.208 | 0.968 | 1.248 | 0.813 | 1.791 | 1.497 | 1.203 | 0.840 | 1.431 | 0.814 | 2.090 | 2.559 | 1.569 | 2.145 | 1.367 |
| Std Dev | 0.0E+00 | 1.8E-03 | 1.6E-03 | 2.2E-03 | 1.9E-03 | 6.1E-04 | 4.5E-04 | 5.5E-04 | 5.5E-04 | 9.6E-04 | 9.6E-04 | 9.1E-04 | 5.5E-04 | 4.5E-04 | 4.5E-04 | 1.5E-03 | 1.8E-03 | 1.3E-04 |
| %RSD | 0.00 | 0.10 | 0.10 | 0.18 | 0.20 | 0.05 | 0.05 | 0.03 | 0.04 | 0.08 | 0.11 | 0.06 | 0.07 | 0.02 | 0.02 | 0.09 | 0.09 | 0.01 |
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| **Nucleoshell HILIC** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | α(2dG/3dG) = k2dG / k3dG | t0 | U | SPTS | k U | k SPTS | α(AX) = kSPTS / kU | t0 | U | TMPAC | k U | k TMPAC | α(CX) = kTMPAC / kU |
| 1 | 0.814 | 3.671 | 3.403 | 3.510 | 3.181 | 1.104 | 0.814 | 1.792 | 0.938 | 1.201 | 0.152 | 0.127 | 0.814 | 1.792 | 5.424 | 1.201 | 5.663 | 4.714 |
| 2 | 0.814 | 3.671 | 3.402 | 3.510 | 3.179 | 1.104 | 0.814 | 1.792 | 0.937 | 1.201 | 0.151 | 0.126 | 0.814 | 1.792 | 5.434 | 1.201 | 5.676 | 4.724 |
| 3 | 0.814 | 3.671 | 3.402 | 3.510 | 3.179 | 1.104 | 0.814 | 1.791 | 0.937 | 1.200 | 0.151 | 0.126 | 0.814 | 1.791 | 5.437 | 1.200 | 5.679 | 4.732 |
| 4 | 0.814 | 3.670 | 3.402 | 3.509 | 3.179 | 1.104 | 0.814 | 1.791 | 0.937 | 1.200 | 0.151 | 0.126 | 0.814 | 1.791 | 5.435 | 1.200 | 5.677 | 4.730 |
| 5 | 0.814 | 3.670 | 3.402 | 3.509 | 3.179 | 1.104 | 0.814 | 1.791 | 0.937 | 1.200 | 0.151 | 0.126 | 0.814 | 1.791 | 5.445 | 1.200 | 5.689 | 4.740 |
| Average | 0.814 | 3.671 | 3.402 | 3.509 | 3.180 | 1.104 | 0.814 | 1.791 | 0.937 | 1.201 | 0.151 | 0.126 | 0.814 | 1.791 | 5.435 | 1.201 | 5.677 | 4.728 |
| Std Dev | 0.0E+00 | 5.5E-04 | 4.5E-04 | 6.7E-04 | 5.5E-04 | 2.2E-04 | 0.0E+00 | 5.5E-04 | 4.5E-04 | 6.7E-04 | 5.5E-04 | 4.2E-04 | 0.0E+00 | 5.5E-04 | 7.5E-03 | 6.7E-04 | 9.2E-03 | 9.8E-03 |
| %RSD | 0.00 | 0.01 | 0.01 | 0.02 | 0.02 | 0.02 | 0.00 | 0.03 | 0.05 | 0.06 | 0.36 | 0.33 | 0.00 | 0.03 | 0.14 | 0.06 | 0.16 | 0.21 |
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| **Nucleoshell HILIC** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | |
| Retention times (min) | | | Retention factors | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | α(Tb/Tp) = kTb / kTp |
| 1 | 0.814 | 1.140 | 1.125 | 0.400 | 0.382 | 1.048 |
| 2 | 0.814 | 1.141 | 1.126 | 0.402 | 0.383 | 1.048 |
| 3 | 0.814 | 1.141 | 1.126 | 0.402 | 0.383 | 1.048 |
| 4 | 0.814 | 1.141 | 1.126 | 0.402 | 0.383 | 1.048 |
| 5 | 0.814 | 1.141 | 1.125 | 0.402 | 0.382 | 1.051 |
| Average | 0.814 | 1.141 | 1.126 | 0.401 | 0.383 | 1.049 |
| Std Dev | 0.0E+00 | 4.5E-04 | 5.5E-04 | 5.5E-04 | 6.7E-04 | 1.5E-03 |
| %RSD | 0.00 | 0.04 | 0.05 | 0.14 | 0.18 | 0.14 |

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| **HILIC-Z** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | | | |
| Retention times (min) | | | Retention factors | | | Parameter | Retention times (min) | | | | Retention factors | | | Parameter | | Retention times (min) | | | | Retention factors | | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) = kU/ k5MU | | t0 | U | 2dU | k U | | k 2dU | α(OH) = kU / k2dU | | t0 | | A | V | k A | | k V | α(V/A) = kV / kA | |
| 1 | 0.410 | 1.096 | 0.944 | 1.673 | 1.302 | 1.285 | | 0.411 | 1.097 | 0.863 | 1.669 | | 1.100 | 1.518 | | 0.410 | | 1.181 | 1.490 | 1.880 | | 2.634 | 1.401 | |
| 2 | 0.410 | 1.096 | 0.944 | 1.673 | 1.302 | 1.285 | | 0.411 | 1.097 | 0.864 | 1.669 | | 1.102 | 1.514 | | 0.411 | | 1.182 | 1.491 | 1.876 | | 2.628 | 1.401 | |
| 3 | 0.411 | 1.096 | 0.945 | 1.667 | 1.299 | 1.283 | | 0.411 | 1.098 | 0.864 | 1.672 | | 1.102 | 1.517 | | 0.411 | | 1.182 | 1.491 | 1.876 | | 2.628 | 1.401 | |
| 4 | 0.411 | 1.096 | 0.945 | 1.667 | 1.299 | 1.283 | | 0.411 | 1.098 | 0.864 | 1.672 | | 1.102 | 1.517 | | 0.411 | | 1.182 | 1.491 | 1.876 | | 2.628 | 1.401 | |
| 5 | 0.411 | 1.097 | 0.945 | 1.669 | 1.299 | 1.285 | | 0.411 | 1.097 | 0.864 | 1.669 | | 1.102 | 1.514 | | 0.411 | | 1.182 | 1.491 | 1.876 | | 2.628 | 1.401 | |
| Average | 0.411 | 1.096 | 0.945 | 1.670 | 1.301 | 1.284 | | 0.411 | 1.097 | 0.864 | 1.670 | | 1.102 | 1.516 | | 0.411 | | 1.182 | 1.491 | 1.877 | | 2.629 | 1.401 | |
| Std Dev | 5.5E-04 | 4.5E-04 | 5.5E-04 | 3.3E-03 | 1.7E-03 | 1.0E-03 | | 0.0E+00 | 5.5E-04 | 4.5E-04 | 1.3E-03 | | 1.1E-03 | 1.5E-03 | | 4.5E-04 | | 4.5E-04 | 4.5E-04 | 2.0E-03 | | 2.9E-03 | 0.0E+00 | |
| %RSD | 0.13 | 0.04 | 0.06 | 0.20 | 0.13 | 0.08 | | 0.00 | 0.05 | 0.05 | 0.08 | | 0.10 | 0.10 | | 0.11 | | 0.04 | 0.03 | 0.11 | | 0.11 | 0.00 | |
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| **HILIC-Z** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | | | |
| Retention times (min) | | | Retention factors | | | Parameter | Retention times (min) | | | | Retention factors | | | Parameter | | Retention times (min) | | | | Retention factors | | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | α(2dG/3dG) = k2dG / k3dG | | t0 | U | SPTS | k U | | k SPTS | α(AX) = kSPTS / kU | | t0 | | U | TMPAC | k U | | k TMPAC | α(CX) = kTMPAC / kU | |
| 1 | 0.411 | 2.261 | 2.102 | 4.501 | 4.114 | 1.094 | | 0.412 | 1.096 | 0.683 | 1.660 | | 0.658 | 0.396 | | 0.411 | | 1.096 | 1.594 | 1.667 | | 2.878 | 1.727 | |
| 2 | 0.411 | 2.261 | 2.102 | 4.501 | 4.114 | 1.094 | | 0.411 | 1.096 | 0.683 | 1.667 | | 0.662 | 0.397 | | 0.411 | | 1.096 | 1.593 | 1.667 | | 2.876 | 1.726 | |
| 3 | 0.411 | 2.262 | 2.103 | 4.504 | 4.117 | 1.094 | | 0.412 | 1.096 | 0.683 | 1.660 | | 0.658 | 0.396 | | 0.412 | | 1.096 | 1.594 | 1.660 | | 2.869 | 1.728 | |
| 4 | 0.411 | 2.262 | 2.103 | 4.504 | 4.117 | 1.094 | | 0.412 | 1.096 | 0.683 | 1.660 | | 0.658 | 0.396 | | 0.411 | | 1.096 | 1.594 | 1.667 | | 2.878 | 1.727 | |
| 5 | 0.411 | 2.262 | 2.103 | 4.504 | 4.117 | 1.094 | | 0.412 | 1.097 | 0.683 | 1.663 | | 0.658 | 0.396 | | 0.411 | | 1.097 | 1.593 | 1.669 | | 2.876 | 1.723 | |
| Average | 0.411 | 2.262 | 2.103 | 4.503 | 4.116 | 1.094 | | 0.412 | 1.096 | 0.683 | 1.662 | | 0.659 | 0.396 | | 0.411 | | 1.096 | 1.594 | 1.666 | | 2.875 | 1.726 | |
| Std Dev | 0.0E+00 | 5.5E-04 | 5.5E-04 | 1.3E-03 | 1.3E-03 | 0.0E+00 | | 4.5E-04 | 4.5E-04 | 0.0E+00 | 2.8E-03 | | 1.8E-03 | 5.2E-04 | | 4.5E-04 | | 4.5E-04 | 5.5E-04 | 3.3E-03 | | 3.9E-03 | 2.0E-03 | |
| %RSD | 0.00 | 0.02 | 0.03 | 0.03 | 0.03 | 0.00 | | 0.11 | 0.04 | 0.00 | 0.17 | | 0.27 | 0.13 | | 0.11 | | 0.04 | 0.03 | 0.20 | | 0.13 | 0.11 | |
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| **HILIC-Z** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | | |
| Retention times (min) | | | Retention factors | | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | α(Tb/Tp) = kTb / kTp | |
| 1 | 0.411 | 0.584 | 0.584 | 0.421 | 0.421 | 1.000 | |
| 2 | 0.412 | 0.584 | 0.584 | 0.417 | 0.417 | 1.000 | |
| 3 | 0.411 | 0.584 | 0.584 | 0.421 | 0.421 | 1.000 | |
| 4 | 0.411 | 0.584 | 0.584 | 0.421 | 0.421 | 1.000 | |
| 5 | 0.412 | 0.584 | 0.584 | 0.417 | 0.417 | 1.000 | |
| Average | 0.411 | 0.584 | 0.584 | 0.420 | 0.420 | 1.000 | |
| Std Dev | 5.5E-04 | 0.0E+00 | 0.0E+00 | 1.9E-03 | 1.9E-03 | 0.0E+00 | |
| %RSD | 0.13 | 0.00 | 0.00 | 0.45 | 0.45 | 0.00 | |

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| **Syncronis HILIC** | Mix1: t0, uridine (U), 5-methyluridine (5MU) | | | | | | | Mix2: t0, uridine (U), 2’-deoxyuridine (2dU) | | | | | | Mix3: t0, adenosine (A), vidarabine (V) | | | | | | | |
| Retention times (min) | | | Retention factors | | Parameter | | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | | | Retention factors | | Parameter |
| Injection No. | t0 | U | 5MU | k U | k 5MU | α(CH3) = kU/ k5MU | | t0 | U | 2dU | k U | k 2dU | α(OH) = kU / k2dU | t0 | A | | V | | k A | k V | α(V/A) = kV / kA |
| 1 | 0.484 | 1.255 | 1.101 | 1.593 | 1.275 | 1.250 | | 0.484 | 1.254 | 1.011 | 1.591 | 1.089 | 1.461 | 0.484 | 1.405 | | 1.767 | | 1.903 | 2.651 | 1.393 |
| 2 | 0.484 | 1.256 | 1.102 | 1.595 | 1.277 | 1.249 | | 0.485 | 1.255 | 1.012 | 1.588 | 1.087 | 1.461 | 0.484 | 1.405 | | 1.767 | | 1.903 | 2.651 | 1.393 |
| 3 | 0.484 | 1.255 | 1.101 | 1.593 | 1.275 | 1.250 | | 0.484 | 1.255 | 1.011 | 1.593 | 1.089 | 1.463 | 0.484 | 1.405 | | 1.766 | | 1.903 | 2.649 | 1.392 |
| 4 | 0.484 | 1.255 | 1.101 | 1.593 | 1.275 | 1.250 | | 0.484 | 1.255 | 1.011 | 1.593 | 1.089 | 1.463 | 0.484 | 1.405 | | 1.766 | | 1.903 | 2.649 | 1.392 |
| 5 | 0.484 | 1.255 | 1.101 | 1.593 | 1.275 | 1.250 | | 0.484 | 1.254 | 1.011 | 1.591 | 1.089 | 1.461 | 0.484 | 1.406 | | 1.767 | | 1.905 | 2.651 | 1.392 |
| Average | 0.484 | 1.255 | 1.101 | 1.593 | 1.275 | 1.250 | | 0.484 | 1.255 | 1.011 | 1.591 | 1.088 | 1.462 | 0.484 | 1.405 | | 1.767 | | 1.903 | 2.650 | 1.392 |
| Std Dev | 0.0E+00 | 4.5E-04 | 4.5E-04 | 9.2E-04 | 9.2E-04 | 1.8E-04 | | 4.5E-04 | 5.5E-04 | 4.5E-04 | 2.2E-03 | 1.0E-03 | 1.0E-03 | 0.0E+00 | 4.5E-04 | | 5.5E-04 | | 9.2E-04 | 1.1E-03 | 6.9E-04 |
| %RSD | 0.00 | 0.04 | 0.04 | 0.06 | 0.07 | 0.01 | | 0.09 | 0.04 | 0.04 | 0.14 | 0.09 | 0.07 | 0.00 | 0.03 | | 0.03 | | 0.05 | 0.04 | 0.05 |
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| **Syncronis HILIC** | Mix4: t0, 2’ deoxyguanosine (2d), 3’- deoxyguanosine (3d) | | | | | | | Mix5: t0, uridine (U), sodium p-toluenesulfonate (SPTS) | | | | | | Mix6: t0, uridine (U), N,N,N-trimethylphenylammonium chloride (TMPAC) | | | | | | | |
| Retention times (min) | | | Retention factors | | | Parameter | Retention times (min) | | | Retention factors | | Parameter | Retention times (min) | | | | | Retention factors | | Parameter |
| Injection No. | t0 | 2dG | 3dG | k 2dG | k 3dG | | α(2dG/3dG) = k2dG / k3dG | t0 | U | SPTS | k U | k SPTS | α(AX) = kSPTS / kU | t0 | | U | | TMPAC | k U | k TMPAC | α(CX) = kTMPAC / kU |
| 1 | 0.485 | 2.665 | 2.481 | 4.495 | 4.115 | | 1.092 | 0.484 | 1.254 | 0.63 | 1.591 | 0.302 | 0.190 | 0.484 | | 1.254 | | 3.144 | 1.591 | 5.496 | 3.455 |
| 2 | 0.485 | 2.665 | 2.480 | 4.495 | 4.113 | | 1.093 | 0.484 | 1.255 | 0.63 | 1.593 | 0.302 | 0.189 | 0.484 | | 1.255 | | 3.144 | 1.593 | 5.496 | 3.450 |
| 3 | 0.485 | 2.665 | 2.480 | 4.495 | 4.113 | | 1.093 | 0.484 | 1.255 | 0.63 | 1.593 | 0.302 | 0.189 | 0.484 | | 1.255 | | 3.145 | 1.593 | 5.498 | 3.451 |
| 4 | 0.485 | 2.665 | 2.480 | 4.495 | 4.113 | | 1.093 | 0.485 | 1.255 | 0.63 | 1.588 | 0.299 | 0.188 | 0.484 | | 1.255 | | 3.145 | 1.593 | 5.498 | 3.451 |
| 5 | 0.485 | 2.665 | 2.480 | 4.495 | 4.113 | | 1.093 | 0.484 | 1.254 | 0.63 | 1.591 | 0.302 | 0.190 | 0.484 | | 1.254 | | 3.146 | 1.591 | 5.500 | 3.457 |
| Average | 0.485 | 2.665 | 2.480 | 4.495 | 4.114 | | 1.093 | 0.484 | 1.255 | 0.630 | 1.591 | 0.301 | 0.189 | 0.484 | | 1.255 | | 3.145 | 1.592 | 5.498 | 3.453 |
| Std Dev | 0.0E+00 | 0.0E+00 | 4.5E-04 | 0.0E+00 | 9.2E-04 | | 2.4E-04 | 4.5E-04 | 5.5E-04 | 0.0E+00 | 2.2E-03 | 1.2E-03 | 5.4E-04 | 0.0E+00 | | 5.5E-04 | | 8.4E-04 | 1.1E-03 | 1.7E-03 | 2.9E-03 |
| %RSD | 0.00 | 0.00 | 0.02 | 0.00 | 0.02 | | 0.02 | 0.09 | 0.04 | 0.00 | 0.14 | 0.40 | 0.29 | 0.00 | | 0.04 | | 0.03 | 0.07 | 0.03 | 0.08 |
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| **Syncronis HILIC** | Mix7: t0, theobromine (Tb), theophylline (Tp) | | | | | | |
| Retention times (min) | | | Retention factors | | | Parameter |
| Injection No. | t0 | Tb | Tp | k Tb | k Tp | | α(Tb/Tp) = kTb / kTp |
| 1 | 0.484 | 0.712 | 0.712 | 0.471 | 0.471 | | 1.000 |
| 2 | 0.484 | 0.712 | 0.712 | 0.471 | 0.471 | | 1.000 |
| 3 | 0.484 | 0.711 | 0.711 | 0.469 | 0.469 | | 1.000 |
| 4 | 0.484 | 0.711 | 0.711 | 0.469 | 0.469 | | 1.000 |
| 5 | 0.484 | 0.711 | 0.711 | 0.469 | 0.469 | | 1.000 |
| Average | 0.484 | 0.711 | 0.711 | 0.470 | 0.470 | | 1.000 |
| Std Dev | 0.0E+00 | 5.5E-04 | 5.5E-04 | 1.1E-03 | 1.1E-03 | | 0.0E+00 |
| %RSD | 0.00 | 0.08 | 0.08 | 0.24 | 0.24 | | 0.00 |