**Supplementary file**

**Table S 1** Chromatographic conditions and range investigated during experimental design

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Unit** | **Type** | **SD** | **Low Value** | **High Value** |
| Methanol  in mobile phase | % | variable | 0 | 40 | 50 |
| Flow rate | min/mL | variable | 0 | 0.8 | 1.2 |
| Resolution1 | Min | response | 0.200 | 3.1 | 4.7 |
| Resolution2 | Min | response | 0.406 | 5.2 | 7.6 |
| Resolution3 | Min | response | 0.422 | 3.1 | 5.3 |

**Table S 2** Table of suggested experimental design and their responses

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Run** | **Variable 1**  **A:Methanol in**  **mobile phase**  **(%)** | **Variable 2**  **B:Flow Rate**  **(min/mL)** | **Response-1**  **Resolution1**  **(min)** |  | **Response-2**  **Resolution2**  **(min)** | **Response-3**  **Resolution3**  **(min)** |
| 1 | 45.00 | 1.00 | 4.11 |  | 6.38 | 3.96 |
| 2 | 50.00 | 1.20 | 3.24 |  | 5.74 | 3.71 |
| 3 | 45.00 | 1.00 | 4.07 |  | 6.30 | 3.87 |
| 4 | 53.00 | 1.00 | 3.17 |  | 5.21 | 3.22 |
| 5 | 40.00 | 1.00 | 4.30 |  | 7.62 | 4.26 |
| 6 | 50.00 | 0.80 | 3.70 |  | 6.39 | 3.87 |
| 7 | 37.53 | 1.00 | 4.32 |  | 6.81 | 4.80 |
| 8 | 45.00 | 1.28 | 3.36 |  | 5.25 | 3.20 |
| 9 | 50.00 | 1.00 | 3.65 |  | 5.93 | 4.03 |
| 10 | 40.00 | 0.80 | 4.35 |  | 7.01 | 5.30 |
| 11 | 45.00 | 0.72 | 4.32 |  | 6.75 | 4.05 |
| 12 | 43.00 | 1.00 | 4.79 |  | 5.99 | 3.12 |
| 13 | 40.00 | 1.23 | 3.76 |  | 5.82 | 3.22 |

**Table S 3** ANOVA results for response Y (Resolution -1) obtained from experimental design

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | SS | Df | MS | Model P  Value | Model F  Value | Prob> F |
| %of methanol  mobile phase | 1.32 | 1 | 0.553 | 0.0018 | 13.31 | Significant |
| Flow Rate | 0.679 | 1 | 0.679 |

SS: Sum of square, df: degree of freedom, MS: Mean Square

**Table S 4** ANOVA results for response Y (Resolution 2) obtained from experimental design

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | SS | Df | MS | Model P  Value | Model F  Value | Prob > F |
| %methanol  mobile phase | 2.15 | 1 | 2.052 | 0.0019 | 12.44 | Significant |
| Flow rate | 2.01 | 1 | 2.01 |

SS: Sum of square, df: degree of freedom, MS: Mean Square

**Table S 5** ANOVA results for response Y (Resolution 3) obtained from experimental design

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | SS | Df | MS | Model P  Value | Model F  Value | Prob> F |
| %acetonitrile  mobile phase | 1.06 | 1 | 1.06 | 0.013 | 6.363 | Significant |
| Flow rate | 1.42 | 1 | 1.42 |

SS: Sum of square, df: degree of freedom, MS: Mean Square