**Table S1** Chromatographic conditions and range investigated during experimental design

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Unit** | **Type** | **SD** | **Low Value** | **High Value** |
| Acetonitrile  in mobile phase | % | variable | 0 | 34 | 44 |
| Flow rate | min/mL | variable | 0 | 0.5 | 1.2 |
| Resolution | min | response | 0.099 | 6.42 | 7.68 |
| Tailing factor | % | response | 0.030 | 0.89 | 1.31 |

**Table S2** Table of suggested experimental design and their responses

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Run** | **Factor -1**  **A:Acetonitrile**  **mobile phase**  **(%)** | **Factor 2**  **B:Flow Rate**  **(min/ml)** | **Response-1**  **Resolution**  **(min)** | **Response-2 Tailing Factor  ( %)** |
| 1 | 42.00 | 0.85 | 7.58 | 1.02 |
| 2 | 39.00 | 0.50 | 7.53 | 0.91 |
| 3 | 39.00 | 0.85 | 7.5 | 1.09 |
| 4 | 42.00 | 1.00 | 7.6 | 1.00 |
| 5 | 39.00 | 1.20 | 7.49 | 1.11 |
| 6 | 39.00 | 0.85 | 7.51 | 1.05 |
| 7 | 39.00 | 0.85 | 7.52 | 1.02 |
| 8 | 34.00 | 0.85 | 6.42 | 1.09 |
| 9 | 34.00 | 1.20 | 6.6 | 1.31 |
| 10 | 34.00 | 0.50 | 6.8 | 0.9 |
| 11 | 39.00 | 0.85 | 7.65 | 1.01 |
| 12 | 42.00 | 0.50 | 7.68 | 0.89 |
| 13 | 39.00 | 0.85 | 7.4 | 1.04 |

**Table S3** ANOVA results for response Y (resolution) obtained from experimental design

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | SS | df | MS | Model P  Value | Model F  Value | Prob > F |
| % of acetonitrile  mobile phase | 0.414 | 1 | 0.414 | 0.0001 | 42.34 | Significant |
| Flow Rate | 0.0039 | 1 | 0.0039 |

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

**Table S4** ANOVA results for response Y (tailing factor) obtained from experimental design

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | SS | df | MS | Model P  Value | Model F  Value | Prob> F |
| % of acetonitrile  mobile phase | 0.012 | 1 | 0.029 | 0.0001 | 29.25 | Significant |
| Flow rate | 0.048 | 1 | 0.048 |

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