Supplementary Data

Tables 1 - 14

Figures 1- 14
Table 1.

| Code | Variable       | Low | High |
|------|----------------|-----|------|
| A    | SGP (psig)     | 22  | 50   |
| B    | AGP (psig)     | 5   | 10   |
| C    | SWGP (psig)    | 0.7 | 1.3  |
| D    | VT (°C)        | 220 | 320  |
| E    | ITT (°C)       | 215 | 266  |
Table 2.

| Run | SGP | AGP | SWGP | VT   | ITT$^1$ |
|-----|-----|-----|------|------|---------|
| 1   | 50  | 10  | 1.3  | 320  | 215     |
| 2   | 22  | 10  | 0.7  | 320  | 215     |
| 3   | 50  | 10  | 0.7  | 220  | 266     |
| 4   | 36  | 7.5 | 1    | 270  | 240.5   |
| 5   | 36  | 7.5 | 1    | 270  | 240.5   |
| 6   | 50  | 10  | 0.7  | 220  | 215     |
| 7   | 22  | 5   | 0.7  | 220  | 266     |
| 8   | 50  | 5   | 0.7  | 220  | 266     |
| 9   | 22  | 5   | 1.3  | 320  | 215     |
| 10  | 22  | 5   | 0.7  | 320  | 266     |
| 11  | 22  | 10  | 1.3  | 320  | 215     |
| 12  | 36  | 7.5 | 1    | 270  | 240.5   |
| 13  | 36  | 7.5 | 1    | 270  | 301.15  |
| 14  | 36  | 7.5 | 1    | 270  | 240.5   |
| 15  | 50  | 5   | 0.7  | 320  | 215     |
| 16  | 36  | 7.5 | 1    | 270  | 240.5   |
| 17  | 36  | 7.5 | 1.71352 | 270 | 240.5 |
| 18  | 36  | 7.5 | 1    | 270  | 179.85  |
| 19  | 22  | 5   | 1.3  | 320  | 266     |
| 20  | 50  | 10  | 1.3  | 220  | 215     |
| 21  | 22  | 5   | 1.3  | 220  | 215     |
| 22  | 22  | 10  | 1.3  | 320  | 266     |
| 23  | 50  | 5   | 1.3  | 220  | 266     |
| 24  | 2.7022 | 7.5 | 1    | 270  | 240.5   |
| 25  | 36  | 13.446 | 1    | 270  | 240.5   |

| Run | SGP | AGP | SWGP | VT   | ITT$^1$ |
|-----|-----|-----|------|------|---------|
| 26  | 50  | 5   | 0.7  | 320  | 266     |
| 27  | 22  | 10  | 0.7  | 220  | 266     |
| 28  | 50  | 10  | 0.7  | 320  | 215     |
| 29  | 50  | 5   | 1.3  | 320  | 266     |
| 30  | 50  | 10  | 1.3  | 320  | 266     |
| 31  | 22  | 5   | 0.7  | 220  | 215     |
| 32  | 22  | 10  | 0.7  | 320  | 266     |
| 33  | 22  | 5   | 1.3  | 220  | 266     |
| 34  | 50  | 10  | 0.7  | 320  | 266     |
| 35  | 50  | 5   | 1.3  | 220  | 215     |
| 36  | 50  | 10  | 1.3  | 220  | 266     |
| 37  | 36  | 7.5 | 1    | 388.921 | 240.5 |
| 38  | 36  | 7.5 | 1    | 270  | 240.5   |
| 39  | 36  | 1.55396 | 1    | 270 | 240.5 |
| 40  | 22  | 10  | 0.7  | 220  | 215     |
| 41  | 50  | 5   | 0.7  | 220  | 215     |
| 42  | 36  | 7.5 | 1    | 270  | 240.5   |
| 43  | 36  | 7.5 | 0.28648 | 270 | 240.5 |
| 44  | 36  | 7.5 | 1    | 151.079 | 240.5 |
| 45  | 22  | 5   | 0.7  | 320  | 215     |
| 46  | 69.2978 | 7.5 | 1    | 270  | 240.5   |
| 47  | 50  | 5   | 1.3  | 320  | 215     |
| 48  | 22  | 10  | 1.3  | 220  | 215     |
| 49  | 36  | 7.5 | 1    | 270  | 240.5   |
| 50  | 22  | 10  | 1.3  | 220  | 266     |

$^1$ Nearest integer selected; $^2$ First decimal value selected; $^3$ input value 2 instead 1.55396 was recommended by LC-ESI-MS software.
| Run | Response-I | Response-II | Response-III | Mean | RSD (%) |
|-----|------------|-------------|--------------|------|---------|
| 1   | 138688     | 138237      | 132962       | 136629 | 2.33    |
| 2   | 107162     | 105454      | 104124       | 105580 | 1.44    |
| 3   | 124797     | 124937      | 123837       | 124523.67 | 0.48    |
| 4   | 130171     | 129308      | 131364       | 130281 | 0.79    |
| 5   | 127741     | 131147      | 129105       | 129331 | 1.32    |
| 6   | 102457     | 105903      | 107444       | 105268 | 2.42    |
| 7   | 100877     | 102924      | 100528       | 101443 | 1.27    |
| 8   | 121175     | 124125      | 124425       | 123241.67 | 1.45    |
| 9   | 95359      | 95054       | 93897        | 94770  | 0.81    |
| 10  | 107369     | 104369      | 104225       | 105321 | 1.68    |
| 11  | 93723      | 100418      | 94442        | 96194.333 | 3.82    |
| 12  | 115925     | 120266      | 118910       | 118367 | 1.87    |
| 13  | 155991     | 156563      | 158249       | 156934.33 | 0.74    |
| 14  | 116680     | 119114      | 118790       | 118194.67 | 1.11    |
| 15  | 116896     | 121687      | 115038       | 117873.67 | 2.91    |
| 16  | 116075     | 119564      | 117352       | 117663.67 | 1.50    |
| 17  | 118974     | 118658      | 121261       | 119631 | 1.18    |
| 18  | 101134     | 99598       | 102397       | 101043 | 1.38    |
| 19  | 105640     | 100633      | 100959       | 102410.67 | 2.73    |
| 20  | 100312     | 99818       | 106727       | 102285.67 | 3.76    |
| 21  | 86382      | 85623       | 84928        | 85644.333 | 0.84    |
| 22  | 99579      | 102173      | 101419       | 101057 | 1.32    |
| 23  | 117967     | 113120      | 116168       | 115751.67 | 2.11    |
| 24  | 18925      | 17548       | 19495        | 18656  | 5.36    |
| 25  | 106692     | 105650      | 107959       | 106767 | 1.08    |

| Run | Response-I | Response-II | Response-III | Mean | RSD (%) |
|-----|------------|-------------|--------------|------|---------|
| 26  | 130753     | 140994      | 135668       | 135805 | 3.77    |
| 27  | 93827      | 92452       | 98439        | 94906  | 3.30    |
| 28  | 119470     | 120294      | 121547       | 120437 | 0.86    |
| 29  | 142446     | 139344      | 138934       | 140241.33 | 1.36    |
| 30  | 122092     | 131584      | 134221       | 129299 | 4.93    |
| 31  | 87321      | 84661       | 85524        | 85835.333 | 1.58    |
| 32  | 106024     | 108568      | 106391       | 106994.33 | 1.28    |
| 33  | 94461      | 96672       | 93727        | 94953.333 | 1.61    |
| 34  | 132536     | 136580      | 127591       | 132235.67 | 3.40    |
| 35  | 108851     | 100619      | 105489       | 104986.33 | 3.94    |
| 36  | 116801     | 116172      | 113761       | 115578 | 1.38    |
| 37  | 146296     | 148590      | 144858       | 146581.33 | 1.28    |
| 38  | 120713     | 123016      | 121203       | 121644 | 0.99    |
| 39  | 121052     | 121722      | 121661       | 121478.33 | 0.30    |
| 40  | 86285      | 85612       | 84885        | 85594  | 0.81    |
| 41  | 102594     | 100364      | 102503       | 101820.33 | 1.23    |
| 42  | 124993     | 125894      | 123366       | 124751 | 1.02    |
| 43  | 118229     | 123890      | 121726       | 121281.67 | 2.35    |
| 44  | 108613     | 109143      | 107666       | 108474 | 0.68    |
| 45  | 93315      | 92871       | 93831        | 93339  | 0.51    |
| 46  | 111130     | 110703      | 112644       | 11492.33 | 0.91    |
| 47  | 123545     | 116864      | 124485       | 121631.33 | 3.41    |
| 48  | 86033      | 88335       | 86113        | 86827  | 1.50    |
| 49  | 126764     | 129238      | 126497       | 127499.67 | 1.18    |
| 50  | 95219      | 96726       | 98377        | 96774  | 1.63    |
Figure 1.

1A: Internally Studentized Residuals vs. Normal % Probability

1B: Internally Studentized Residuals vs. Predicted

1C: Internally Studentized Residuals vs. Run Number
Figure 2.

2 A

2 B

2 C

2 D
### A)

| Source   | Sequential p-value | Lack of Fit p-value | Adjusted R-Squared | Predicted R-Squared |
|----------|--------------------|---------------------|--------------------|---------------------|
| Linear   | 1.70959E-07        | 0.005869154         | 0.53584903         | 0.446291918         |
| 2FI      | 0.996977414        | 0.002742207         | 0.42888809         | 0.381168838         |
| Quadratic| 5.61767E-09        | 0.152542858         | 0.8582919          | 0.678961949         |
| Cubic    | 0.141081908        | 0.264045063         | 0.89952894         | -0.283328963        |

### B)

| Source                      | Sum of Squares     | df  | Mean Square  | F Value     | p-value  | Prob > F     |
|-----------------------------|--------------------|-----|--------------|-------------|----------|--------------|
| Mean vs Total               | 6.15174E+11        | 1   | 6.15174E+11  |             |          |              |
| Linear vs Mean              | 12468051551        | 5   | 2493610310   | 12.31381991 | 1.70959E-07|              |
| 2FI vs Linear               | 438401949.8        | 10  | 43840194.98  | 0.17594411  | 0.996977414|              |
| Quadratic vs 2FI            | 6678864594         | 5   | 1335772919   | 21.60535643 | 5.61767E-09| Suggested    |
| Cubic vs Quadratic          | 1179269154         | 15  | 78617943.58  | 1.793511523 | 0.141081908| Aliased      |
| Residual                   | 613685050.9        | 14  | 43834646.49  |             |          |              |
| Total                      | 6.36553E+11        | 50  | 12731051434  |             |          |              |

### C)

| Source      | Sum of Squares     | df  | Mean Square  | F Value     | p-value (Prob > F) |
|-------------|--------------------|-----|--------------|-------------|--------------------|
| Linear      | 8678044643         | 37  | 234541747.1  | 7.071322978 | 0.005869154        |
| 2FI         | 8239642693         | 27  | 305171951.6  | 9.200790308 | 0.002742207        |
| Quadratic   | 1560778099         | 22  | 70944459.05  | 2.138941956 | 0.152542858        |
| Cubic       | 381508945.4        | 7   | 54501277.91  | 1.643187806 | 0.264045063        |
| Pure Error  | 232176105.5        | 7   | 33168015.07  |             |                    |

### D)

| Source      | Std.Dev.       | R-Squared    | Adjusted R-Squared | Predicted R-Squared | PRESS     |
|-------------|----------------|--------------|--------------------|---------------------|-----------|
| Linear      | 14230.42575    | 0.583211374  | 0.53584903         | 0.446291918         | 11837322146|
| 2FI         | 15785.15572    | 0.603718267  | 0.42888809         | 0.381168838         | 13229541099|
| Quadratic   | 7862.951548    | 0.916131941  | 0.8582919          | 0.678961949         | 6863238881 Suggested|
| Cubic       | 6620.773859    | 0.971293983  | 0.89952894         | -0.283328963        | 27435356026 Aliased |
| Source  | Sum of Squares | df | Mean Square  | F value | p-value  | Prob > F |
|---------|----------------|----|--------------|---------|----------|----------|
| Model   | 1.96E+10       | 20 | 9.79E+08     | 15.83906| 1.23E-10 | Significant |
| A-SGP   | 8.37E+09       | 1  | 8.37E+09     | 135.3782| 1.91E-12 | Significant |
| B-AGP   | 44900623       | 1  | 44900623     | 0.726242| 0.401087 | Not significant |
| C-SWGP  | 367200         | 1  | 367200       | 0.005939| 0.9391   | Not significant |
| D-VT    | 2.06E+09       | 1  | 2.06E+09     | 33.35416| 2.96E-06 | Significant |
| E-ITT   | 1.99E+09       | 1  | 1.99E+09     | 32.19895| 3.9E-06  | Significant |
| AB      | 551775.1       | 1  | 551775.1     | 0.008925| 0.925385 | Not significant |
| AC      | 65574152       | 1  | 65574152     | 1.060624| 0.311586 | Not significant |
| AD      | 90135451       | 1  | 90135451     | 1.457889| 0.237022 | Not significant |
| AE      | 25095070       | 1  | 25095070     | 0.405898| 0.529057 | Not significant |
| BC      | 37113420       | 1  | 37113420     | 0.600288| 0.44474  | Not significant |
| BD      | 9954722        | 1  | 9954722      | 0.161012| 0.691168 | Not significant |
| BE      | 77128200       | 1  | 77128200     | 1.247504| 0.273198 | Not significant |
| CD      | 13590291       | 1  | 13590291     | 0.219815| 0.642686 | Not significant |
| CE      | 51374316       | 1  | 51374316     | 0.83095 | 0.369514 | Not significant |
| DE      | 67884552       | 1  | 67884552     | 1.097993| 0.303362 | Not significant |
| A²      | 6.25E+09       | 1  | 6.25E+09     | 101.0249| 5.88E-11 | Significant |
| B²      | 2.15E+08       | 1  | 2.15E+08     | 3.478703| 0.072311 | Not significant |
| C²      | 71115500       | 1  | 71115500     | 1.150252| 0.292341 | Not significant |
| D²      | 10453927       | 1  | 10453927     | 0.169086| 0.683948 | Not significant |
| E²      | 22027506       | 1  | 22027506     | 0.356282| 0.55521  | Not significant |
| Residual| 1.79E+09       | 29 | 61826007     |         |          | Not significant |
| Lack of Fit | 1.56E+09   | 22 | 70944459     | 2.138942| 0.152543 | Not significant |
| Pure Error | 2.32E+08  | 7  | 33168015     |         |          | Not significant |
Figure 4.
Figure 5.
Figure 6.
Table 6.

| Prediction Run | SGP | AGP | SWGP | VT  | ITT  | Predicted Area | Experimental Area |
|----------------|-----|-----|------|-----|------|----------------|-------------------|
| 1              | 22  | 7.5 | 1.006| 272 | 242.54| 98013.67       | 118584.67         |
| 2              | 36  | 7.5 | 1.006| 272 | 242.54| 122683.23      | 150743.67         |
| 3              | 50  | 7.5 | 1.006| 272 | 242.54| 126148.99      | 144819.67         |
| 4              | 50  | 7.5 | 1.006| 320 | 242.54| 134717.89      | 150587.33         |
| 5              | 50  | 7.5 | 1.006| 320 | 266  | 141031.99      | 155813.67         |
| 6              | 50  | 7.5 | 1.3   | 320 | 266  | 140610.74      | 153123.00         |
| 7              | 25  | 7.5 | 1    | 270 | 240.5 | 104401.31      | 126092.67         |
| 8              | 45  | 7.5 | 1    | 270 | 240.5 | 126423.61      | 132912.67         |
| 9              | 45  | 7.5 | 1    | 300 | 240.5 | 131367.12      | 136567.00         |
| 10             | 10  | 5   | 0.5  | 220 | 220   | 48397.23       | 47715.33          |
| 11             | 10  | 5   | 0.2  | 240 | 240   | 48397.23       | 57027.67          |
| 12             | 5   | 5   | 0.5  | 200 | 200   | 21936.21       | 21332.33          |
| 13             | 7.5 | 5   | 0.5  | 270 | 250   | 51797.44       | 37862.00          |
| 14             | 10  | 10  | 2    | 235 | 200   | 28057.52       | 47108.50          |
| 15             | 2   | 5   | 0.4  | 245 | 220   | 17211.29       | 14213.00          |
| 16             | 30  | 10  | 0.7  | 260 | 240   | 110360.39      | 108005.00         |
| 17             | 45  | 5   | 0.3  | 280 | 220   | 107260.31      | 114636.00         |
| 18             | 35  | 8   | 0.7  | 270 | 250   | 122765.45      | 126415.67         |
Table 7.

| Code | Variable                  | Low | High |
|------|---------------------------|-----|------|
| A    | Column Temp (°C)          | 30  | 55   |
| B    | Flow Rate (ml/min)        | 0.25| 0.3  |
| C    | %B at 3min                | 30  | 65   |
| D    | %B at 4.1min              | 30  | 65   |
| E    | %B at 6min                | 30  | 65   |
| Run | Column Temp (°C) | Flow Rate (ml/min) | %B at 3min | %B at 4.1min | %B at 6min |
|-----|------------------|--------------------|------------|--------------|------------|
| 1   | 42.5             | 0.275              | 89.12      | 47.5         | 47.5       |
| 2   | 55               | 0.3                | 65         | 65           | 30         |
| 3   | 55               | 0.3                | 65         | 30           | 65         |
| 4   | 55               | 0.3                | 65         | 65           | 65         |
| 5   | 55               | 0.3                | 65         | 30           | 30         |
| 6   | 30               | 0.3                | 65         | 30           | 30         |
| 7   | 30               | 0.3                | 65         | 65           | 65         |
| 8   | 30               | 0.3                | 65         | 65           | 30         |
| 9   | 30               | 0.3                | 65         | 30           | 65         |
| 10  | 55               | 0.25               | 65         | 65           | 65         |
| 11  | 55               | 0.25               | 65         | 30           | 30         |
| 12  | 55               | 0.25               | 65         | 65           | 30         |
| 13  | 55               | 0.25               | 65         | 30           | 65         |
| 14  | 30               | 0.25               | 65         | 65           | 65         |
| 15  | 30               | 0.25               | 65         | 65           | 30         |
| 16  | 30               | 0.25               | 65         | 30           | 30         |
| 17  | 30               | 0.25               | 65         | 30           | 65         |
| 18  | 42.5             | 0.33               | 47.5       | 47.5         | 47.5       |
| 19  | **72.23**        | **0.275**          | **47.5**   | **47.5**     | **47.5**   |
| 20  | 42.5             | 0.275              | 47.5       | 47.5         | 47.5       |
| 21  | 42.5             | 0.275              | 47.5       | 47.5         | 47.5       |
| 22  | 42.5             | 0.275              | 47.5       | 47.5         | 47.5       |
| 23  | 42.5             | 0.275              | 47.5       | 47.5         | 5.87       |
| 24  | 42.5             | 0.275              | 47.5       | 47.5         | 47.5       |
| 25  | 42.5             | 0.275              | 47.5       | 5.87         | 47.5       |

Table 8.
| Run | Response-1 | Response-2 | Response-3 | Average   | % RSD |
|-----|------------|------------|------------|-----------|-------|
| 1   | 82932      | 86111      | 86744      | 85262.33  | 2.40  |
| 2   | 78973      | 84240      | 80699      | 81304.00  | 3.30  |
| 3   | 81303      | 85590      | 87199      | 84697.33  | 3.60  |
| 4   | 86208      | 81061      | 84074      | 83781.00  | 3.09  |
| 5   | 90216      | 88064      | 97067      | 91782.33  | 5.12  |
| 6   | 74021      | 72828      | 74105      | 73651.33  | 0.97  |
| 7   | 72556      | 67535      | 68734      | 69608.33  | 3.77  |
| 8   | 74367      | 69842      | 69560      | 71256.33  | 3.79  |
| 9   | 73469      | 70939      | 70373      | 71593.67  | 2.30  |
| 10  | 87045      | 96733      | 93993      | 92590.33  | 5.39  |
| 11  | 102574     | 105280     | 106035     | 104629.67 | 1.74  |
| 12  | 105642     | 105491     | 104898     | 105343.67 | 0.37  |
| 13  | 110338     | 103202     | 102923     | 105487.67 | 3.98  |
| 14  | 84716      | 79978      | 78809      | 81167.67  | 3.85  |
| 15  | 80266      | 79475      | 82214      | 80651.67  | 1.75  |
| 16  | 89543      | 88485      | 86216      | 88081.33  | 1.93  |
| 17  | 82185      | 83127      | 79644      | 81652.00  | 2.21  |
| 18  | 82062      | 81976      | 79972      | 81336.67  | 1.45  |
| 19  | 124903     | 127382     | 135592     | 129292.33 | 4.33  |
| 20  | 93022      | 92334      | 88091      | 91149.00  | 2.93  |
| 21  | 90661      | 90635      | 91268      | 90854.67  | 0.39  |
| 22  | 89903      | 90693      | 91724      | 90773.33  | 1.01  |
| 23  | 94554      | 91908      | 105806     | 97422.67  | 7.57  |
| 24  | 94331      | 96247      | 91018      | 93865.33  | 2.82  |
| 25  | 97811      | 97305      | 99250      | 98122.00  | 1.03  |

| Run | Response-1 | Response-2 | Response-3 | Average   | % RSD |
|-----|------------|------------|------------|-----------|-------|
| 26  | 91419      | 87881      | 88821      | 89373.67  | 2.05  |
| 27  | 87172      | 90085      | 94071      | 90442.67  | 3.83  |
| 28  | 94613      | 91629      | 93715      | 93319.00  | 1.64  |
| 29  | 95573      | 99410      | 95531      | 96838.00  | 2.30  |
| 30  | 84751      | 88884      | 88155      | 87263.33  | 2.53  |
| 31  | 88154      | 84730      | 88786      | 87223.33  | 2.50  |
| 32  | 79498      | 80641      | 79640      | 79926.33  | 0.78  |
| 33  | 108418     | 109105     | 109454     | 108992.33 | 0.48  |
| 34  | 107622     | 107172     | 105943     | 106912.33 | 0.81  |
| 35  | 111176     | 101900     | 105753     | 106276.33 | 4.38  |
| 36  | 99759      | 105890     | 99765      | 101804.67 | 3.48  |
| 37  | 114982     | 112762     | 110500     | 112748.00 | 1.99  |
| 38  | 87651      | 89161      | 88777      | 88529.67  | 0.89  |
| 39  | 89301      | 91764      | 91235      | 90766.67  | 1.43  |
| 40  | 79587      | 79064      | 78825      | 79158.67  | 0.49  |
| 41  | 81832      | 85386      | 86509      | 84575.67  | 2.89  |
| 42  | 122309     | 129132     | 131065     | 127502.00 | 3.61  |
| 43  | 119134     | 110448     | 112285     | 113955.67 | 4.02  |
| 44  | 122209     | 123820     | 126598     | 124209.00 | 1.79  |
| 45  | 123251     | 121678     | 127939     | 124289.33 | 2.62  |
| 46  | 102855     | 102108     | 100002     | 101655.00 | 1.46  |
| 47  | 94616      | 90741      | 92578      | 92645.00  | 2.09  |
| 48  | 98164      | 99762      | 94304      | 97410.00  | 2.88  |
| 49  | 93638      | 92723      | 86939      | 91100.00  | 3.99  |
| 50  | 106568     | 102875     | 105888     | 105110.33 | 1.87  |
### Table 10.

#### A)

| Source      | Sequential p-value | Lack of Fit p-value | Adjusted R-Squared | Predicted R-Squared |
|-------------|--------------------|---------------------|--------------------|---------------------|
| Linear      | 3.04826E-22        | 0.087736713         | 0.903670243        | 0.88553389          |
| 2FI         | 0.366964338        | 0.08916514          | 0.90651774         | 0.877699184         |
| Quadratic   | 0.011241656        | 0.19337112          | 0.93263729         | 0.852448935         |
| Cubic       | 0.301197544        | 0.210339295         | 0.942375665        | 0.425126156         |

#### B)

| Source          | Sum of Squares   | df | Mean Square   | F Value      | p-value Prob > F |
|-----------------|------------------|----|--------------|--------------|-----------------|
| Mean vs Total   | 4.43189E+11      | 1  | 4.43189E+11  |              |                 |
| Linear vs Mean  | 9153523641       | 5  | 1830704728   | 92.93388053  | 3.04826E-22     |
| 2FI vs Linear   | 216788234.5      | 10 | 21678823.45  | 1.134025286  | 0.366964338     |
| Quadratic vs 2FI| 250482097.1      | 5  | 50096419.43  | 3.63666561   | 0.011241656     |
| Cubic vs Quadratic | 234510925.6 | 15 | 15634061.71  | 1.326728714  | 0.301197544     |
| Residual        | 164974844.9      | 14 | 11783917.49  |              |                 |
| Total           | 4.53209E+11      | 50 | 9064189989   |              |                 |

#### C)

| Source          | Sum of Squares   | df | Mean Square   | F Value      | p-value Prob > F |
|-----------------|------------------|----|--------------|--------------|-----------------|
| Linear          | 809642496.8      | 37 | 21882229.64  | 2.681946037  | 0.087736713     |
| 2FI             | 592854262.3      | 27 | 21957565.27  | 2.691179378  | 0.08916514      |
| Quadratic       | 342372165.2      | 22 | 15562371.14  | 1.907366859  | 0.193371112     |
| Cubic           | 107861239.6      | 7  | 15408748.51  | 1.888538448  | 0.210339295     |
| Pure Error      | 57113605.33      | 7  | 8159086.476  |              |                 |

#### D)

| Source          | Std.Dev.         | R-Squared   | Adjusted R-Squared | Predicted R-Squared | PRESS          |
|-----------------|------------------|-------------|--------------------|---------------------|----------------|
| Linear          | 4438.355813      | 0.91349981  | 0.903670243        | 0.88553389          | 1146982441     |
| 2FI             | 4372.265087      | 0.935134758 | 0.90651774         | 0.877699184         | 1225488392     |
| Quadratic       | 3711.518745      | 0.960132274 | 0.93263729         | 0.852448935         | 1478502949     |
| Cubic           | 3432.77111       | 0.983535904 | 0.942375665        | 0.425126156         | 5760396737     |
Table 11.

### ANOVA for Response Surface Quadratic model

| Source       | Sum of Squares | df | Mean Square | F value | p-value | Prob > F |
|--------------|----------------|----|-------------|---------|---------|----------|
| Model        | 9.626E+09      | 21 | 4.584E+08   | 32.54   | < 0.0001| Significant|
| A-Column Temp | 4.495E+09      | 1  | 4.495E+09   | 319.07  | < 0.0001| Significant|
| B-Flow Rate  | 1.806E+09      | 1  | 1.806E+09   | 128.22  | < 0.0001| Significant|
| C-%B at 3min | 2.416E+09      | 1  | 2.416E+09   | 171.48  | < 0.0001| Significant|
| D-%B at 4.1min | 1.766E+08    | 1  | 1.766E+08   | 12.54   | 0.0014  | Significant|
| E-%B at 6min | 2.605E+08      | 1  | 2.605E+08   | 18.49   | 0.0002  | Significant|
| AB           | 5.908E+07      | 1  | 5.908E+07   | 4.19    | 0.0501  | Not significant|
| AC           | 1.121E+08      | 1  | 1.121E+08   | 7.96    | 0.0087  | Significant|
| AD           | 7.208E+06      | 1  | 7.208E+06   | 0.51    | 0.4803  | Not significant|
| AE           | 3.4300.17      | 1  | 3.4300.17   | 2.435E+03 | 0.9610 | Not significant|
| BC           | 3.085E+06      | 1  | 3.085E+06   | 0.22    | 0.6434  | Not significant|
| BD           | 20418.84       | 1  | 20418.84    | 1.450E+03 | 0.9699 | Not significant|
| BE           | 3.878E+06      | 1  | 3.878E+06   | 0.28    | 0.6039  | Not significant|
| CD           | 8.066E+05      | 1  | 8.066E+05   | 0.057   | 0.8126  | Not significant|
| CE           | 2.776E+07      | 1  | 2.776E+07   | 1.97    | 0.1714  | Not significant|
| DE           | 2.783E+06      | 1  | 2.783E+06   | 0.20    | 0.6601  | Not significant|
| A²           | 2.389E+08      | 1  | 2.389E+08   | 16.96   | 0.0003  | Significant|
| B²           | 9.065E+06      | 1  | 9.065E+06   | 0.64    | 0.4292  | Not significant|
| C²           | 9.239E+06      | 1  | 9.239E+06   | 0.66    | 0.4249  | Not significant|
| D²           | 1.309E+06      | 1  | 1.309E+06   | 0.093   | 0.7628  | Not significant|
| E²           | 5.003E+05      | 1  | 5.003E+05   | 0.036   | 0.8519  | Not significant|
| Residual     | 3.944E+08      | 28 | 1.409E+07   |         |         |          |
| Lack of Fit  | 3.373E+08      | 21 | 1.606E+07   | 1.97    | 0.1818  | Not significant|
| Pure Error   | 5.711E+07      | 7  | 8.159E+06   |         |         |          |
Figure 9

9 A

9 B

9 C

9 D
Figure. 10

**10 A**

Area (counts*min)

Flow rate

Column temperature

**10 B**

Area (counts*min)

% B at 3 min

Column temperature

**10 C**

Area (counts*min)

% B at 4.1 min

Column temperature

**10 D**

Area (counts*min)

% B at 6 min

Column temperature
Figure. 11

11 A Area (counts*min)

11 B Area (counts*min)

11 C Area (counts*min)
Figure. 12

12 A  
Area (counts*min)

12 B  
Area (counts*min)
Figure. 13

Area (counts*min)

%B at 4.1min

%B at 6min

88000

90000

92000

94000

80
| Prediction Run | Column Temp (°C) | Flow Rate (mL/min) | %B at 3min | %B at 4.1min | %B at 6min | Predicted Area | Experimental Area (EA) | EA %RSD |
|---------------|-----------------|-------------------|-------------|--------------|-------------|----------------|-----------------------|---------|
| 1             | 70              | 0.22              | 6           | 85           | 6           | 187464.01       | 143542.00             | 2.34    |
| 2             | 70              | 0.25              | 10          | 85           | 10          | 172043.19       | 134888.00             | 2.84    |
| 3             | 70              | 0.3               | 10          | 85           | 20          | 150306.58       | 124599.33             | 1.52    |
| 4             | 72              | 0.275             | 47.5        | 47.5         | 47.5        | 156284.32       | 135711.00             | 2.01    |
| 5             | 60              | 0.275             | 20          | 85           | 15          | 140603.19       | 119764.33             | 1.34    |
| 6             | 50              | 0.22              | 10          | 85           | 10          | 148162.10       | 137895.66             | 5.72    |
| 7             | 40              | 0.25              | 35          | 85           | 35          | 112932.47       | 120031.33             | 1.33    |
| 8             | 30              | 0.25              | 15          | 85           | 15          | 111274.78       | 113412.00             | 3.43    |
| 9             | 30              | 0.25              | 35          | 85           | 35          | 102438.18       | 112510.00             | 0.92    |
| 10            | 30              | 0.25              | 6           | 85           | 6           | 116311.67       | 116579.00             | 1.79    |
Unpaired t test

P value summary  *

Are means signif. different? (P < 0.05)  Yes
One- or two-tailed P value? Two-tailed

P value  0.0350
t, df t=3.136 df=4

Figure. 14
## Table 13

| Method    | VT  | ITT | SGP | AGP | SWGP |
|-----------|-----|-----|-----|-----|------|
| Gen-MS-I  | 250 | 200 | 25  | 2   | 0.5  |
| CCD-MS    | 270 | 301 | 36  | 7.5 | 1    |
| CCD-LC    | 270 | 301 | 36  | 7.5 | 1    |

## Table 14

| Method    | Column Temperature | Flow rate (ml/min) | Time (min) | %B (Acetonitrile) |
|-----------|--------------------|--------------------|------------|-------------------|
| Gen-MS-I  | 30                 | 0.25               | 0 to 4     | 50 to 80          |
|           |                    | 0.25               | 4 to 8     | 80 to 50          |
|           |                    | 0.25               | 8 to 15    | 50 to 50          |
| CCD-MS    | 30                 | 0.25               | 0 to 4     | 50 to 80          |
|           |                    | 0.25               | 4 to 8     | 80 to 50          |
|           |                    | 0.25               | 8 to 15    | 50 to 50          |
| CCD-LC    | 70                 | 0.25               | 0 to 3     | 50 to 6           |
|           |                    | 0.22               | 3 to 4.1   | 6 to 85           |
|           |                    | 0.22               | 4.1 to 6   | 85 to 6           |
|           |                    | 0.25               | 6 to 8     | 6 to 50           |