

# Comprehensive Analysis of Metabolites in Brews Prepared from Naturally and Technologically Treated Coffee Beans

Magdalena Jeszka-Skowron, Robert Frankowski , Agnieszka Zgoła-Grze'skowiak and Julia Płatkiewicz

## Supplementary Tables

Supplementary Table S1. LC mobile phase gradient and MS source parameters

| Mobile phase gradient<br>(percentage of acetonitrile)                              | Parameters of source  |
|--|---|
| 0 min 7%,<br>2 min 15%,<br>5 min 40%,<br>6 min 60%<br>6.5 min 100%<br>8.6 min 100% | curtain gas 20 psi<br>nebulizer gas 45 psi<br>auxiliary gas 45 psi<br>temperature 450 °C<br>collision gas medium<br>ion spray voltage 4500 V<br>entrance potential 10 V |

Supplementary Table S2. Mass spectrometer parameters applied for the determination of compounds

| Compound                | DP <sup>a</sup> [V] | Analytical transition | CE <sup>b</sup> [eV] | EP <sup>c</sup> [V] | CXP <sup>d</sup> [V] | Confirmatory transition | CE [eV] | EP [V] | CXP [V] |
|-------------------------|---------------------|-----------------------|----------------------|---------------------|----------------------|-------------------------|---------|--------|---------|
| Trigonelline            | 50                  | 138 → 94              | 30                   | 10                  | 4                    | 138 → 110               | 30      | 10     | 5       |
| Theanine                | 35                  | 175 → 158             | 17                   | 10                  | 9                    | 175 → 84                | 30      | 10     | 4       |
| Quinic acid             | 60                  | 193 → 147             | 13                   | 5                   | 9                    | 193 → 111               | 21      | 5      | 5       |
| Nicotinamide            | 20                  | 123 → 80              | 28                   | 10                  | 2                    | 123 → 96                | 28      | 10     | 4       |
| Nicotinic acid          | 30                  | 124 → 80              | 30                   | 10                  | 3                    | 124 → 78                | 32      | 10     | 3       |
| Serotonin               | 50                  | 177 → 160             | 9                    | 10                  | 12                   | 177 → 132               | 31      | 6      | 7       |
| Theobromine             | 40                  | 181 → 110             | 31                   | 10                  | 5                    | 181 → 138               | 25      | 10     | 7       |
| Theophylline            | 50                  | 181 → 96              | 33                   | 10                  | 4                    | 181 → 124               | 27      | 10     | 6       |
| 5-CQA                   | 50                  | 355 → 163             | 18                   | 5                   | 10                   | 355 → 145               | 45      | 3      | 7       |
| Caffeine                | 25                  | 195 → 138             | 26                   | 10                  | 5                    | 195 → 110               | 32      | 10     | 5       |
| 4-CQA                   | 50                  | 355 → 163             | 18                   | 5                   | 10                   | 355 → 145               | 45      | 3      | 7       |
| 3-CQA                   | 50                  | 355 → 163             | 18                   | 5                   | 10                   | 355 → 145               | 45      | 3      | 7       |
| Caffeic acid            | 48                  | 181 → 163             | 15                   | 8                   | 9                    | 181 → 135               | 27      | 6      | 7       |
| Melatonin               | 85                  | 233 → 174             | 19                   | 5                   | 10                   | 233 → 159               | 40      | 6      | 10      |
| Ferulic acid            | 45                  | 195 → 177             | 15                   | 4                   | 10                   | 195 → 145               | 23      | 7      | 7       |
| <i>p</i> -Coumaric acid | 45                  | 165 → 147             | 15                   | 3                   | 7                    | 165 → 119               | 25      | 4      | 6       |
| 3,4-diCQA               | 55                  | 517 → 163             | 26                   | 5                   | 11                   | 517 → 499               | 13      | 5      | 15      |
| 3,5-diCQA               | 55                  | 517 → 163             | 26                   | 5                   | 11                   | 517 → 499               | 13      | 5      | 15      |
| 4,5-diCQA               | 55                  | 517 → 163             | 26                   | 5                   | 11                   | 517 → 499               | 13      | 5      | 15      |

<sup>a</sup>DP – declustering potential, <sup>b</sup>CE – collision energy, <sup>c</sup>EP – cell entrance potential, <sup>d</sup>CXP – cell exit potential

Supplementary Table S3. LC-MS/MS parameters for analyzed compounds (RT – retention time; LOD – limit of detection; LOQ – limit of quantification)

| Compound                | RT [min] | Intercept | Slope     | R <sup>2</sup> | Range [ $\mu\text{g mL}^{-1}$ ] |       | LOD [ $\mu\text{g mL}^{-1}$ ] | LOQ [ $\mu\text{g/mL}^{-1}$ ] |
|-------------------------|----------|-----------|-----------|----------------|---------------------------------|-------|-------------------------------|-------------------------------|
|                         |          |           |           |                | min                             | max   |                               |                               |
| Trigonelline            | 0.73     | 66728     | 42979115  | 0.9985         | 0.001                           | 0.05  | 0.00002                       | 0.00006                       |
| Theanine                | 0.76     | 22629     | 21128693  | 0.9994         | 0.0005                          | 0.2   | 0.00004                       | 0.00014                       |
| Quinic acid             | 0.77     | 2123      | 345922    | 0.9992         | 0.0025                          | 1.0   | 0.00465                       | 0.01551                       |
| Nicotinamide            | 0.91     | 36020     | 39248609  | 0.9996         | 0.0005                          | 0.05  | 0.00001                       | 0.00005                       |
| Nicotinic acid          | 0.92     | 7691      | 22552053  | 0.9998         | 0.0005                          | 0.05  | 0.00006                       | 0.00021                       |
| Serotonin               | 0.97     | 14241     | 22007112  | 0.9993         | 0.0005                          | 0.1   | 0.00003                       | 0.00008                       |
| Theobromine             | 1.42     | -61.64    | 4336884   | 1.0000         | 0.0005                          | 0.5   | 0.00009                       | 0.0003                        |
| Theophylline            | 2.02     | 10859     | 6508748   | 0.9991         | 0.0005                          | 0.2   | 0.00012                       | 0.0004                        |
| 5-CQA                   | 2.53     | 1786      | 781478    | 1.0000         | 0.0025                          | 2.5   | 0.00047                       | 0.00156                       |
| Caffeine                | 2.61     | 43041     | 18229109  | 0.9992         | 0.001                           | 0.2   | 0.00004                       | 0.00012                       |
| 4-CQA                   | 3.42     | 4750      | 551201    | 0.9996         | 0.0025                          | 2.5   | 0.00072                       | 0.00239                       |
| 3-CQA                   | 3.70     | 6861      | 3902774   | 0.9999         | 0.002                           | 1.0   | 0.0001                        | 0.00034                       |
| Caffeic acid            | 4.36     | 8294      | 7322094   | 0.9998         | 0.0005                          | 0.5   | 0.0007                        | 0.00233                       |
| Melatonin               | 5.02     | 48058     | 135783791 | 0.9992         | 0.00025                         | 0.025 | 0.00000106                    | 0.00000353                    |
| Ferulic acid            | 5.1      | 25627     | 12881257  | 0.9998         | 0.0005                          | 0.5   | 0.00044                       | 0.00148                       |
| <i>p</i> -Coumaric acid | 5.38     | 14546     | 7028294   | 0.9997         | 0.0005                          | 0.5   | 0.00043                       | 0.00144                       |
| 3,4-diCQA               | 5.32     | 5745      | 221049    | 0.9993         | 0.005                           | 2.5   | 0.00125                       | 0.00416                       |
| 3,5-diCQA               | 5.53     | -3227     | 246328    | 0.9998         | 0.005                           | 2.5   | 0.00149                       | 0.00497                       |
| 4,5-diCQA               | 5.69     | 709       | 270530    | 0.9995         | 0.005                           | 0.5   | 0.00102                       | 0.00341                       |

|  |                |          |             |          |                  |  |  |  |  |
|--|----------------|----------|-------------|----------|------------------|--|--|--|--|
| Coefficient  | t for H0       |          |             | R-       |                  |  |  |  |  |
| Removed  | Estimate       | Coeff=0  | Prob >  t   | Squared  | MSE              |  |  |  |  |
| B  | 2090.00        | 0.81     | 0.4392      | 0.1271   | 3.258E+007       |  |  |  |  |
| A  | 3230.00        | 1.27     | 0.2319      | 0.0000   | 3.421E+007       |  |  |  |  |
| ANOVA for Response Surface Mean Model – nicotinic acid         |                |          |             |          |                  |  |  |  |  |
| Analysis of variance table [Partial sum of squares - Type III] |                |          |             |          |                  |  |  |  |  |
| Source   | Sum of Squares | df       | Mean Square | F Value  | p-value Prob > F |  |  |  |  |
| Model  | 0.000          | 0        |             |          |                  |  |  |  |  |
| Residual   | 4.106E+008     | 12       | 3.421E+007  |          |                  |  |  |  |  |
| Lack of Fit  | 2.933E+008     | 8        | 3.666E+007  | 1.25     | 0.4419           |  |  |  |  |
| Pure Error   | 1.173E+008     | 4        | 2.931E+007  |          |                  |  |  |  |  |
| Cor Total  | 4.106E+008     | 12       |             |          |                  |  |  |  |  |
|  | Coefficient    | t for H0 |             | R-       |                  |  |  |  |  |
| Removed  | Estimate       | Coeff=0  | Prob >  t   | Squared  | MSE              |  |  |  |  |
| B  | 92000          | 0,198752 | 0,8464      | 0,196802 | 9,78E+11         |  |  |  |  |
| A  | 726000         | 1,641724 | 0,1289      | 1,89E-15 | 1,12E+12         |  |  |  |  |
| ANOVA for Response Surface Mean Model - 4-CQA                  |                |          |             |          |                  |  |  |  |  |
| Analysis of variance table [Partial sum of squares - Type III] |                |          |             |          |                  |  |  |  |  |

| Source      | Sum of Squares | df | Mean Square | F Value | p-value Prob > F |                 |
|-------------|----------------|----|-------------|---------|------------------|-----------------|
| Model       |                | 0  | 0           |         |                  |                 |
| Residual    | 1,3391E+13     | 12 | 1,11592E+12 |         |                  |                 |
| Lack of Fit | 6,62E+12       | 8  | 8,275E+11   | 0.48885 | 0.8195           | not significant |
| Pure Error  | 6,771E+12      | 4  | 1,69275E+12 |         |                  |                 |
| Cor Total   | 1,3391E+13     | 12 |             |         |                  |                 |

| ANOVA for Response Surface Mean Model – 3-CQA                  |                |    |             |         |                  | Std. Dev.       | 2.146E+006 | R-Squared      | 0.0000  |
|--|----------------|----|-------------|---------|------------------|-----------------|------------|----------------|---------|
| Analysis of variance table [Partial sum of squares - Type III] |                |    |             |         |                  | Mean            | 4.530E+007 | Adj R-Squared  | 0.0000  |
|  |                |    |             |         |                  | C.V. %          | 4.74       | Pred R-Squared | -0.1736 |
|  |                |    |             |         |                  | PRESS           | 6.483E+013 | Adeq Precision |         |
| Source   | Sum of Squares | df | Mean Square | F Value | p-value Prob > F |                 |            |                |         |
| Model  | 0.000          | 0  |             |         |                  |                 |            |                |         |
| Residual   | 5.524E+013     | 12 | 4.603E+012  |         |                  |                 |            |                |         |
| Lack of Fit  | 4.725E+013     | 8  | 5.906E+012  | 2.96    | 0.1549           | not significant |            |                |         |
| Pure Error   | 7.988E+012     | 4  | 1.997E+012  |         |                  |                 |            |                |         |
| Cor Total  | 5.524E+013     | 12 |             |         |                  |                 |            |                |         |

Supplementary Table S5. ANOVA for Response Surface Models Arabica Brazil Mogiana roasted coffee

|  |             |    |             |          |          |                 |           |             |                |          |
|--|-------------|----|-------------|----------|----------|-----------------|-----------|-------------|----------------|----------|
| <b>ANOVA for Response Surface Linear Model - caffeine</b>      |             |    |             |          |          |                 | Std. Dev. | 59230,31968 | R-Squared      | 0,623145 |
| Analysis of variance table [Partial sum of squares - Type III] |             |    |             |          |          |                 | Mean      | 1480769,231 | Adj R-Squared  | 0,547774 |
|  | Sum of      |    | Mean        | F        | p-value  |                 | C.V. %    | 3,999969641 | Pred R-Squared | 0,0123   |
| Source   | Squares     | df | Square      | Value    | Prob > F |                 | PRESS     | 91947306733 | Adeq Precision | 10,68416 |
| Model  | 58010000000 | 2  | 29005000000 | 8,2677   | 0.0076   | significant     |           |             |                |          |
| A-Temperature  | 25205000000 | 1  | 25205000000 | 7,184533 | 0.0231   |                 |           |             |                |          |
| B-Time   | 32805000000 | 1  | 32805000000 | 9,350867 | 0.0121   |                 |           |             |                |          |
| Residual   | 35082307692 | 10 | 3508230769  |          |          |                 |           |             |                |          |
| Lack of Fit  | 29362307692 | 6  | 4893717949  | 3,42218  | 0.1269   | not significant |           |             |                |          |
| Pure Error   | 5720000000  | 4  | 1430000000  |          |          |                 |           |             |                |          |
| Cor Total  | 93092307692 | 12 |             |          |          |                 |           |             |                |          |
| <b>ANOVA for Response Surface Mean Model – ferulic acid</b>    |             |    |             |          |          |                 | Std. Dev. | 2886,955188 | R-Squared      | 0        |
| Analysis of variance table [Partial sum of squares - Type III] |             |    |             |          |          |                 | Mean      | 6255,384615 | Adj R-Squared  | 0        |
|  | Sum of      |    | Mean        | F        | p-value  |                 | C.V. %    | 46,15152169 | Pred R-Squared | -0,17361 |
| Source   | Squares     | df | Square      | Value    | Prob > F |                 | PRESS     | 117377686,1 | Adeq Precision |          |
| Model  | 0           | 0  |             |          |          |                 |           |             |                |          |
| Residual   | 100014123,1 | 12 | 8334510,256 |          |          |                 |           |             |                |          |
| Lack of Fit  | 50671843,08 | 8  | 6333980,385 | 0,513473 | 0.8041   | not significant |           |             |                |          |
| Pure Error   | 49342280    | 4  | 12335570    |          |          |                 |           |             |                |          |
| Cor Total  | 100014123,1 | 12 |             |          |          |                 |           |             |                |          |

Supplementary Table S6. ANOVA for Response Surface Model Robusta Vietnam green coffee

|  |          |    |             |          |          |                 |           |             |                |          |
|--|----------|----|-------------|----------|----------|-----------------|-----------|-------------|----------------|----------|
| ANOVA for Response Surface Linear Model –<br>Nicotinic acid    |          |    |             |          |          |                 |           |             |                |          |
| Analysis of variance table [Partial sum of squares - Type III] |          |    |             |          |          |                 |           |             |                |          |
|  | Sum of   |    | Mean        | F        | p-value  |                 | Std. Dev. | 1050,877728 | R-Squared      | 0,523077 |
| Source   | Squares  | df | Square      | Value    | Prob > F |                 | Mean      | 12990       | Adj R-Squared  | 0,427692 |
| Model  | 12112160 | 2  | 6056080     | 5,483871 | 0.0247   | significant     | C.V. %    | 8,089897832 | Pred R-Squared | 0,223451 |
| A-Temperature  | 4960080  | 1  | 4960080     | 4,491427 | 0.0601   |                 | PRESS     | 17981458,77 | Adeq Precision | 8,684192 |
| B-Time   | 7152080  | 1  | 7152080     | 6,476315 | 0.0291   |                 |           |             |                |          |
| Residual   | 11043440 | 10 | 1104344     |          |          |                 |           |             |                |          |
| Lack of Fit  | 8583440  | 6  | 1430573,333 | 2,326136 | 0.2167   | not significant |           |             |                |          |
| Pure Error   | 2460000  | 4  | 615000      |          |          |                 |           |             |                |          |
| Cor Total  | 23155600 | 12 |             |          |          |                 |           |             |                |          |

|  |             |    |             |          |          |             |           |             |                |          |
|--|-------------|----|-------------|----------|----------|-------------|-----------|-------------|----------------|----------|
| ANOVA for Response Surface Linear Model -<br>caffeine          |             |    |             |          |          |             |           |             |                |          |
| Analysis of variance table [Partial sum of squares - Type III] |             |    |             |          |          |             |           |             |                |          |
|  | Sum of      |    | Mean        | F        | p-value  |             | Std. Dev. | 79918,22744 | R-Squared      | 0,586257 |
| Source   | Squares     | df | Square      | Value    | Prob > F |             | Mean      | 2301538,462 | Adj R-Squared  | 0,503508 |
| Model  | 90500000000 | 2  | 45250000000 | 7,084789 | 0.0121   | significant | C.V. %    | 3,472382877 | Pred R-Squared | 0,147749 |
|  |             |    |             |          |          |             | PRESS     | 1,31561E+11 | Adeq Precision | 9,898031 |

|  |              |         |              |               |            |                    |            |                   |                    |
|--|--------------|---------|--------------|---------------|------------|--------------------|------------|-------------------|--------------------|
| A-<br>Temperature  | 500000000000 | 1       | 500000000000 | 7,828496      | 0.0189     | not<br>significant |            |                   |                    |
| B-Time   | 405000000000 | 1       | 405000000000 | 6,341082      | 0.0305     |                    |            |                   |                    |
| Residual   | 63869230769  | 10      | 6386923077   |               |            |                    |            |                   |                    |
| Lack of Fit  | 49949230769  | 6       | 8324871795   | 2,392205      | 0.2090     |                    |            |                   |                    |
| Pure Error   | 13920000000  | 4       | 3480000000   |               |            |                    |            |                   |                    |
| Cor Total  | 1,54369E+11  | 12      |              |               |            |                    |            |                   |                    |
| Coefficient  | t for H0     |         |              |               |            | Std. Dev.          | 22451.04   | R-<br>Squared     | 0.8238             |
| Removed  | Estimate     | Coeff=0 | Prob >  t    | R-<br>Squared | MSE        | Mean               | 1.384E+005 | Adj R-<br>Squared | 0.8078             |
| B-Time   | 4070.00      | 0.39    | 0.7051       | 0.8238        | 5.040E+008 | B-Time             | C.V. %     | 16.22             | Pred R-<br>Squared |
|  |              |         |              |               |            | PRESS              | 7.314E+009 | Adeq<br>Precision | 16.355             |
| ANOVA for Response Surface Reduced Linear Model - 4,5-CQA      |              |         |              |               |            |                    |            |                   |                    |
| Analysis of variance table [Partial sum of squares - Type III] |              |         |              |               |            |                    |            |                   |                    |
|  | Sum of       |         | Mean         | F             | p-value    |                    |            |                   |                    |
| Source   | Squares      | df      | Square       | Value         | Prob > F   |                    |            |                   |                    |
| Model  | 2.593E+010   | 1       | 2.593E+010   | 51.44         | < 0.0001   | significant        |            |                   |                    |
| A-<br>Temperature  | 2.593E+010   | 1       | 2.593E+010   | 51.44         | < 0.0001   |                    |            |                   |                    |
| Residual   | 5.545E+009   | 11      | 5.040E+008   |               |            |                    |            |                   |                    |



|             |            |    |            |      |        |                    |
|-------------|------------|----|------------|------|--------|--------------------|
| Lack of Fit | 3.867E+009 | 7  | 5.525E+008 | 1.32 | 0.4165 | not<br>significant |
| Pure Error  | 1.677E+009 | 4  | 4.193E+008 |      |        |                    |
| Cor Total   | 3.147E+010 | 12 |            |      |        |                    |

Supplementary Table S7. ANOVA for Response Surface Models Robusta Vietnam roasted coffee

|  |                |    |             |         |                  |                 |           |            |                |         |
|--|----------------|----|-------------|---------|------------------|-----------------|-----------|------------|----------------|---------|
| <b>ANOVA for Response Surface Mean Model - 5-CQA</b><br>Analysis of variance table [Partial sum of squares - Type III] |                |    |             |         |                  |                 | Std. Dev. | 21202.02   | R-Squared      | 0.0000  |
|  |                |    |             |         |                  |                 | Mean      | 4.858E+005 | Adj R-Squared  | 0.0000  |
|  |                |    |             |         |                  |                 | C.V. %    | 4.36       | Pred R-Squared | -0.1736 |
|  |                |    |             |         |                  |                 | PRESS     | 6.331E+009 | Adeq Precision |         |
| Source   | Sum of Squares | df | Mean Square | F Value | p-value Prob > F |                 |           |            |                |         |
| Model  | 0.000          | 0  |             |         |                  |                 |           |            |                |         |
| Residual   | 5.394E+009     | 12 | 4.495E+008  |         |                  |                 |           |            |                |         |
| Lack of Fit  | 4.725E+009     | 8  | 5.906E+008  | 3.53    | 0.1192           | not significant |           |            |                |         |
| Pure Error   | 6.692E+008     | 4  | 1.673E+008  |         |                  |                 |           |            |                |         |
| Cor Total  | 5.394E+009     | 12 |             |         |                  |                 |           |            |                |         |

|  |                |    |             |          |                  |             |           |             |                |          |
|--|----------------|----|-------------|----------|------------------|-------------|-----------|-------------|----------------|----------|
|  |                |    |             |          |                  |             | Std. Dev. | 6795,206044 | R-Squared      | 0,325398 |
|  |                |    |             |          |                  |             | Mean      | 165076,9231 | Adj R-Squared  | 0,264071 |
|  |                |    |             |          |                  |             | C.V. %    | 4,116387631 | Pred R-Squared | -0,0773  |
|  |                |    |             |          |                  |             | PRESS     | 811126009,2 | Adeq Precision | 5,252694 |
| <b>ANOVA for Response Surface Reduced Linear Model - theobromine</b><br>Analysis of variance table [Partial sum of squares - Type III] |                |    |             |          |                  |             |           |             |                |          |
| Source   | Sum of Squares | df | Mean Square | F Value  | p-value Prob > F |             |           |             |                |          |
| Model  | 245000000      | 1  | 245000000   | 5,305922 | 0.0418           | significant |           |             |                |          |

|             |             |    |             |          |        |                 |
|-------------|-------------|----|-------------|----------|--------|-----------------|
| A-          |             |    |             |          |        |                 |
| Temperature | 245000000   | 1  | 245000000   | 5,305922 | 0.0418 |                 |
| Residual    | 507923076,9 | 11 | 46174825,17 |          |        |                 |
| Lack of Fit | 452723076,9 | 7  | 64674725,27 | 4,686574 | 0.0772 | not significant |
| Pure Error  | 55200000    | 4  | 13800000    |          |        |                 |
| Cor Total   | 752923076,9 | 12 |             |          |        |                 |

| ANOVA for Response Surface Linear Model - Caffeic acid         |                |    |             |          |         |                 | R-        |             |                |          |
|--|----------------|----|-------------|----------|---------|-----------------|-----------|-------------|----------------|----------|
| Analysis of variance table [Partial sum of squares - Type III] |                |    |             |          |         |                 | Std. Dev. | 7512,144014 | Squared        | 0,809795 |
|  |                |    |             |          |         |                 | Mean      | 166923,0769 | Adj R-Squared  | 0,771754 |
|  |                |    |             |          |         |                 | C.V. %    | 4,500362774 | Pred R-Squared | 0,589403 |
|  |                |    |             |          |         |                 | PRESS     | 1218209388  | Adeq Precision | 16,73725 |
| Source   | Sum of Squares | df | Mean Square | F Value  | p-value |                 |           |             |                |          |
| Model  | 2402600000     | 2  | 1201300000  | 21,28745 | 0.0002  | significant     |           |             |                |          |
| A-   |                |    |             |          |         |                 |           |             |                |          |
| Temperature  | 1729800000     | 1  | 1729800000  | 30,65265 | 0.0002  |                 |           |             |                |          |
| B-Time   | 672800000      | 1  | 672800000   | 11,92225 | 0.0062  |                 |           |             |                |          |
| Residual   | 564323076,9    | 10 | 56432307,69 |          |         |                 |           |             |                |          |
| Lack of Fit  | 305523076,9    | 6  | 50920512,82 | 0,787025 | 0.6230  | not significant |           |             |                |          |
| Pure Error   | 258800000      | 4  | 64700000    |          |         |                 |           |             |                |          |
| Cor Total  | 2966923077     | 12 |             |          |         |                 |           |             |                |          |

| ANOVA for Response Surface Mean Model - Quinic acid            |                |    |             |         |         |  | R-        |            |                |         |
|--|----------------|----|-------------|---------|---------|--|-----------|------------|----------------|---------|
| Analysis of variance table [Partial sum of squares - Type III] |                |    |             |         |         |  | Std. Dev. | 20106.13   | Squared        | 0.0000  |
|  |                |    |             |         |         |  | Mean      | 4.264E+005 | Adj R-Squared  | 0.0000  |
|  |                |    |             |         |         |  | C.V. %    | 4.72       | Pred R-Squared | -0.1736 |
| Source   | Sum of Squares | df | Mean Square | F Value | p-value |  |           |            |                |         |

|             |            |    |            |       |          |             |             |            |                |
|-------------|------------|----|------------|-------|----------|-------------|-------------|------------|----------------|
| Source      | Squares    | df | Square     | Value | Prob > F |             | PRESS       | 5.693E+009 | Adeq Precision |
| Model       | 0.000      | 0  |            |       |          |             | Coefficient | Standard   | 95% CI         |
| Residual    | 4.851E+009 | 12 | 4.043E+008 |       |          |             | Factor      | Estimate   | df             |
|             |            |    |            |       |          | not         |             |            | Error          |
| Lack of Fit | 3.798E+009 | 8  | 4.747E+008 | 1.80  | 0.2981   | significant | Intercept   | 4.264E+005 | 1              |
| Pure Error  | 1.053E+009 | 4  | 2.633E+008 |       |          |             |             |            | 5576.44        |
| Cor Total   | 4.851E+009 | 12 |            |       |          |             |             |            |                |