Supporting Materials

Improved achiral and chiral HPLC-UV analysis of ruxolitinib in two different drug formulations

Alessandro Di Michele\textsuperscript{1a}, Aurélie Schoubben\textsuperscript{2a}, Ina Varfaj\textsuperscript{2}, Alessandro D’Arpino\textsuperscript{3}, Laura Mercolini\textsuperscript{4}, Roccaldo Sardella\textsuperscript{2*}, Maurizio Ricci\textsuperscript{2b}, Enrico Tiacci\textsuperscript{5b}

\textsuperscript{1}Department of Physics and Geology, University of Perugia, Via Pascoli 1, 06123 Perugia, Italy
\textsuperscript{2}Department of Pharmaceutical Sciences, University of Perugia, Via Fabretti 48, 06123 – Perugia, Italy
\textsuperscript{3}Hospital Pharmacy Unit, Santa Maria della Misericordia Hospital, Perugia, Italy
\textsuperscript{4}Department of Pharmacy and Biotechnology (FaBiT), Alma Mater Studiorum, University of Bologna, Via Belmeloro 6, 40126 – Bologna (Italy)
\textsuperscript{5}Institute of Haematology and Centre of Haematology-Oncology Research (CREO), University and Hospital of Perugia, Perugia, Italy

Corresponding author:
\textsuperscript{*}Roccaldo Sardella, roccaldosardella@unipg.it

\textsuperscript{a}Alessandro Di Michele and Aurélie Schoubben contributed equally to this work
\textsuperscript{b}Maurizio Ricci and Enrico Tiacci contributed equally to this work
Table S1. Regression data

| Concentration level | Concentration (mg/mL) | Area value (AU) | Mean Area value (AU) | St. Dev. | CV% |
|---------------------|-----------------------|----------------|---------------------|----------|-----|
| 1                   | 0.006                 | 359321         | 349171              | 14080    | 4.0 |
|                     | 0.006                 | 333097         |                     |          |     |
|                     | 0.006                 | 355095         |                     |          |     |
| 2                   | 0.024                 | 1544182        | 1562696.667         | 30645    | 2.0 |
|                     | 0.024                 | 1545838        |                     |          |     |
|                     | 0.024                 | 1598070        |                     |          |     |
| 3                   | 0.04                  | 2349743        | 2351165             | 31978    | 1.4 |
|                     | 0.04                  | 2383830        |                     |          |     |
|                     | 0.04                  | 2319922        |                     |          |     |
| 4                   | 0.06                  | 3279752        | 3279752             | 38360    | 1.2 |
|                     | 0.06                  | 3318112        |                     |          |     |
|                     | 0.06                  | 3241392        |                     |          |     |
| 5                   | 0.1                   | 5052934        | 5008624             | 44310    | 0.9 |
|                     | 0.1                   | 4964314        |                     |          |     |
|                     | 0.1                   | 5008624        |                     |          |     |
| 6                   | 0.11                  | 5492043        | 5522112             | 68073    | 1.2 |
|                     | 0.11                  | 5474251        |                     |          |     |
|                     | 0.11                  | 5600042        |                     |          |     |
| 7                   | 0.12                  | 6078957        | 6133634.333         | 59712    | 1.0 |
|                     | 0.12                  | 6124595        |                     |          |     |
|                     | 0.12                  | 6197351        |                     |          |     |
| 8                   | 0.144                 | 7898542        | 7878717.667         | 93475    | 1.2 |
|                     | 0.144                 | 7776921        |                     |          |     |
|                     | 0.144                 | 7960690        |                     |          |     |
| 9                   | 0.16                  | 9054209        | 8797880             | 254907   | 2.9 |
|                     | 0.16                  | 8795012        |                     |          |     |
|                     | 0.16                  | 8544419        |                     |          |     |
| 10                  | 0.17                  | 9285973        | 9434401.333         | 285371   | 3.0 |
|                     | 0.17                  | 9253835        |                     |          |     |
|                     | 0.17                  | 9763396        |                     |          |     |

7-points regression curve
\[ y = 50907123 (\pm 1256761) + 14852930 (\pm 119637); R^2 = 0.99; R_{xy}^2 = 0.99 \]

10-points regression curve
\[ y = 53947627 (\pm 932149) - 33866 (\pm 101246); R^2 = 0.99; R_{xy}^2 = 0.99 \]
### Table S2 (a). Validation data – Area values

| Concentration (mg/mL) | Area Values (Arbitrary Units, AU) | series 1 | series 2 | series 3 |
|-----------------------|-----------------------------------|----------|----------|----------|
| 0.045                 | 2349743                           | 2313351  | 2367858  |          |
| 0.045                 | 2383830                           | 2353389  | 2366515  |          |
| 0.045                 | 2319922                           | 2388150  | 2355345  |          |
| 0.12                  | 6078957                           | 6489467  | 6143308  |          |
| 0.12                  | 6124595                           | 6420102  | 6336243  |          |
| 0.12                  | 6197351                           | 6485446  | 6232053  |          |
| 0.144                 | 7898542                           | 7883195  | 8066904  |          |
| 0.144                 | 7776921                           | 789698   | 7965940  |          |
| 0.144                 | 7960690                           | 8036829  | 7973943  |          |

### Table S2 (b). Validation data – Calculated concentrations

| Concentration (mg/mL) | Calculated Concentration (mg/mL) | series 1 | series 2 | series 3 |
|-----------------------|-----------------------------------|----------|----------|----------|
| 0.045                 | 0.0432                            | 0.0425   | 0.0436   |          |
| 0.045                 | 0.0439                            | 0.0433   | 0.0436   |          |
| 0.045                 | 0.0427                            | 0.0440   | 0.0433   |          |
| 0.12                  | 0.1165                            | 0.1246   | 0.1178   |          |
| 0.12                  | 0.1174                            | 0.1232   | 0.1215   |          |
| 0.12                  | 0.1188                            | 0.1245   | 0.1195   |          |
| 0.144                 | 0.1522                            | 0.1519   | 0.1555   |          |
| 0.144                 | 0.1498                            | 0.1521   | 0.1536   |          |
| 0.144                 | 0.1535                            | 0.1550   | 0.1537   |          |

### Table S2 (c). Validation data – Recovery%

| Concentration (mg/mL) | Recovery % | series 1 | series 2 | series 3 |
|-----------------------|------------|----------|----------|----------|
| 0.045                 | 96.1       | 94.5     | 96.9     |          |
| 0.045                 | 97.6       | 96.2     | 96.8     |          |
| 0.045                 | 94.8       | 97.8     | 96.3     |          |
| 0.12                  | 97.1       | 103.8    | 98.1     |          |
| 0.12                  | 97.8       | 102.7    | 101.3    |          |
| 0.12                  | 99.0       | 103.7    | 99.6     |          |
| 0.144                 | 105.7      | 105.5    | 108.0    |          |
| 0.144                 | 104.1      | 105.6    | 106.6    |          |
| 0.144                 | 106.6      | 107.6    | 106.7    |          |
Table S2 (d). Validation data – Bias%

| Concentration (mg/mL) | Relative Bias % |
|-----------------------|-----------------|
|                       | series 1 | series 2 | series 3 |
| 0.045                 | -3.9     | -5.5     | -3.1     |
| 0.045                 | -2.4     | -3.8     | -3.2     |
| 0.045                 | -5.2     | -2.2     | -3.7     |
| 0.12                  | -2.9     | 3.8      | -1.9     |
| 0.12                  | -2.2     | 2.7      | 1.3      |
| 0.12                  | -1.0     | 3.7      | -0.4     |
| 0.144                 | 5.7      | 5.5      | 8.0      |
| 0.144                 | 4.1      | 5.6      | 6.6      |
| 0.144                 | 6.6      | 7.6      | 6.7      |
### Table S3. Validation data - Robustness

| Flow-rate (mL/min) | Area (AU)  | Column T (°C) | Area (AU)  |
|-------------------|------------|---------------|------------|
| 1.1               | 5769839    | 26            | 6056125    |
| 1.0               | 5923526    | 25            | 5923526    |
| 0.9               | 5919238    | 24            | 5877975    |
| Average value     | 5870867.7  | Average value | 5952542    |
| St. Dev.          | 87519.657  | St. Dev.      | 92551.62   |
| CV%               | 1.4907448  | CV%           | 1.554825   |
| Concentration (mg/mL) | Area (AU)  | Average Area (AU) | CV% |
|-----------------------|------------|-------------------|-----|
| 0.04                  | 2349743    |                   |     |
|                       | 2383830    |                   |     |
|                       | 2319922    |                   |     |
|                       | 2313351    |                   |     |
|                       | 2353389    | 2355345           | 1.1 |
|                       | 2388150    |                   |     |
|                       | 2367858    |                   |     |
|                       | 2366515    |                   |     |
|                       | 2355345    |                   |     |
| 0.10                  | 5052934    |                   |     |
|                       | 4964314    |                   |     |
|                       | 5008624    |                   |     |
|                       | 5215251    |                   |     |
|                       | 5225879    | 5168820           | 3.1 |
|                       | 5354048    |                   |     |
|                       | 5274271    |                   |     |
|                       | 5392188    |                   |     |
|                       | 5031869    |                   |     |
| 0.12                  | 6078957    |                   |     |
|                       | 6124595    |                   |     |
|                       | 6197351    |                   |     |
|                       | 6489467    |                   |     |
|                       | 6420102    | 6278614           | 2.5 |
|                       | 6485446    |                   |     |
|                       | 6143308    |                   |     |
|                       | 6336243    |                   |     |
|                       | 6232053    |                   |     |
Figure S1. Structure of the Whelk-O1 CSPs employed in the study.

CSP 1; \((R,R)\)-Whelk O1

CSP 2; \((S,S)\)-Whelk O1
Figure S2. Chromatographic analysis of excipients with the optimized RP-HPLC-UV method. (a) Ruxolitinib extracted from the capsule (b) colloidal anhydrous silica, (c) hydroxypropyl cellulose, (d) lactose monohydrate, (e) magnesium stearate, (f) microcrystalline cellulose, (g) povidone.
Figure S3. Plot of CV% vs concentration values in mg/mL. CV% refers to area values (in AU) analyzed in triplicate at each concentration level.