Table S1. Initial risk assessment of the resveratrol nanosuspension.

| CQAs               | Parameters                      | Risk Level | Justification                                                                                                                                 |
|--------------------|--------------------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Resveratrol        | concentration                   | High       | If the concentration of resveratrol is excessively high, particles grow very quickly during the manufacturing process. Therefore, the risk level was high. |
| Stabilizer type    |                                | High       | The ability to inhibit particle growth depends on the type of stabilizer. Therefore, the risk level was high.                                  |
| Stabilizer         | concentration                   | High       | An appropriate concentration of stabilizer has an effective ability to inhibit particle growth. Therefore, the risk level was high.            |
| Solvent type       |                                | High       | The solvent and anti-solvent should be sufficiently miscible, and the solvent should have a solubilization effect. Therefore, the risk level was high. |
| Ratio of solvent/anti-solvent | High       | The solubility of resveratrol for the mixture solvents depends on the ratio of the solvent/anti-solvent. The solubility affects particle growth. Therefore, the risk level was high. |
| Mixing speed       |                                | High       | Depending on the mixing speed, the mixing speed of the anti-solvent and solvent vary, and can affect particle growth rate. Therefore, the risk level was high. |
| Mixing time        |                                | High       | Mixing time can affect particle growth. Therefore, the risk level was high.                                                                  |
| Injection rate (solvent) | High       | Depending on the rate of injection of solvent, the mixing speed of the anti-solvent and solvent vary, which can affect the particle growth rate. Therefore, the risk level was high. |
| Temperature        |                                | High       | The solubility of resveratrol changes when the temperature of the solvent changes, which can affect particle growth. Therefore, the risk level was high. |
| Resveratrol        | concentration                   | Low        | The effect of changes in resveratrol concentration on the zeta potential is insignificant. Therefore, the risk level was low.          |
| Stabilizer type    |                                | High       | Depending on the type of stabilizer, the surface charge of the particles differs. Therefore, the risk level was high.                      |
| Stabilizer         | concentration                   | High       | Depending on the concentration of stabilizer, the surface charge of the particles differs. Therefore, the risk level was high.            |
| Property                        | Level  | Description                                                                                                                                                                                                 |
|--------------------------------|--------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Solvent type                   | Low    | The influence of the type of solvent on the surface charge of the particles was insignificant. Therefore, the risk level was low.                                                                         |
| Ratio of solvent/anti-solvent  | Medium | Depending on the solvent/anti-solvent ratio, the concentration of the stabilizer in the mixture solvent varies, which affects the surface charge of the particles. Therefore, the risk level was medium. |
| Mixing speed                   | Low    | The effect of mixing speed on the zeta potential is insignificant. Therefore, the risk level was low.                                                                                                     |
| Mixing time                    | Low    | The effect of mixing time on the zeta potential is insignificant. Therefore, the risk level was low.                                                                                                       |
| Injection rate (solvent)       | Low    | The effect of injection rate on the zeta potential is insignificant. Therefore, the risk level was low.                                                                                                    |
| Temperature                    | Low    | The effect of temperature on the zeta potential is insignificant. Therefore, the risk level was low.                                                                                                      |
| Drug content                   |        | Resveratrol is chemically stable when light is blocked. Nanosuspensions were prepared in a space where light was blocked, and the possibility of drug loss during the manufacturing process is insignificant. Therefore, the risk level was low. |
| Resveratrol concentration      | Low    |                                                                                                                                                                                                          |
| Stabilizer type                | Low    |                                                                                                                                                                                                          |
| Stabilizer concentration       | Low    |                                                                                                                                                                                                          |
| Solvent type                   | Low    |                                                                                                                                                                                                          |
| Ratio of solvent/anti-solvent  | Low    |                                                                                                                                                                                                          |
| Mixing speed                   | Low    |                                                                                                                                                                                                          |
| Mixing time                    | Low    |                                                                                                                                                                                                          |
| Injection rate (solvent)       | Low    |                                                                                                                                                                                                          |
| Temperature                    | Low    |                                                                                                                                                                                                          |
| Polymer | Concentration (%) | Particle size (z-average, nm) | Particle size (d50, nm) | Particle size (d90, nm) |
|---------|-------------------|------------------------------|------------------------|------------------------|
| PVP K12 | 2.0               | N.D                          | N.D                    | N.D                    |
|         | 1.0               | N.D                          | N.D                    | N.D                    |
|         | 0.5               | N.D                          | N.D                    | N.D                    |
|         | 0.1               | N.D                          | N.D                    | N.D                    |
| PVP K17 | 2.0               | N.D                          | N.D                    | N.D                    |
|         | 1.0               | N.D                          | N.D                    | N.D                    |
|         | 0.5               | N.D                          | N.D                    | N.D                    |
|         | 0.1               | N.D                          | N.D                    | N.D                    |
| PVP K25 | 2.0               | N.D                          | N.D                    | N.D                    |
|         | 1.0               | N.D                          | N.D                    | N.D                    |
|         | 0.5               | N.D                          | N.D                    | N.D                    |
|         | 0.1               | N.D                          | N.D                    | N.D                    |
| PVP K30 | 2.0               | N.D                          | N.D                    | N.D                    |
|         | 1.0               | N.D                          | N.D                    | N.D                    |
|         | 0.5               | N.D                          | N.D                    | N.D                    |
|         | 0.1               | N.D                          | N.D                    | N.D                    |
| PVP K90 | 2.0               | N.D                          | N.D                    | N.D                    |
|         | 1.0               | N.D                          | N.D                    | N.D                    |
|         | 0.5               | N.D                          | N.D                    | N.D                    |
|         | 0.1               | N.D                          | N.D                    | N.D                    |
| PVP VA64| 2.0               | N.D                          | N.D                    | N.D                    |
|         | 1.0               | N.D                          | N.D                    | N.D                    |
|         | 0.5               | N.D                          | N.D                    | N.D                    |
|         | 0.1               | N.D                          | N.D                    | N.D                    |
|          | 2.0  | 1.0  | 0.5  | 0.1  |
|----------|------|------|------|------|
| HPMC 3cp |      |      |      | N.D  |
|          |      |      |      |      |
|          | 515b | 867b | 1357b| N.D  |
|          | 520  | 1004 | 1760 |      |
|          | 6402 | 2034 | 3198 |      |
|          |      |      |      |      |
| HPMC 6cp |      |      |      | N.D  |
|          |      |      |      |      |
|          | 331b | 618b | 978b | N.D  |
|          | 357  | 573  | 1008 |      |
|          | 1290 | 7441 | 1646 |      |
|          |      |      |      |      |
| HPMC 15cp|      |      |      | N.D  |
|          |      |      |      |      |
|          | N.D  | N.D  | N.D  | N.D  |
|          | 663b | 820  | 1337 |      |
|          | N.D  | N.D  | N.D  | N.D  |

*N.D indicates that data cannot be measured and there is no data. *bPrecipitation occurs due to particle agglomeration.
Table S3. Particle size of resveratrol nanosuspensions prepared using a polymer/polymer combination.

| Polymer / Polymer       | Concentration (%, w/v) | Particle size (z-average, nm) | Particle size (d50, nm) | Particle size (d90, nm) |
|-------------------------|------------------------|-------------------------------|------------------------|------------------------|
| PVP VA64 / PVP K12     | 2.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 2.0 / 0.5              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 1.0              | 1151<sup>b</sup>             | 1058                  | 6760                  |
|                         | 0.5                    | N.D                           | N.D                    | N.D                    |
| PVP VA64 / PVP K17     | 2.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 2.0 / 0.5              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 0.5              | 940<sup>b</sup>              | 877                   | 7053                  |
| PVP VA64 / PVP K25     | 2.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 2.0 / 0.5              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 1.0              | 1516<sup>a</sup>             | 1326                  | 2245                  |
|                         | 1.0 / 0.5              | 500<sup>a</sup>              | 572                   | 5782                  |
| PVP VA64 / PVP K30     | 2.0 / 1.0              | 18<sup>b</sup>               | 15                    | 239                    |
|                         | 2.0 / 0.5              | 436<sup>b</sup>              | 196                   | 7521                  |
|                         | 1.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 0.5              | N.D                           | N.D                    | N.D                    |
| PVP VA64 / PVP K90     | 2.0 / 1.0              | 258<sup>b</sup>              | 50                    | 3681                  |
|                         | 2.0 / 0.5              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 1.0              | 948<sup>b</sup>              | 1173                  | 2339                  |
|                         | 1.0 / 0.5              | 1014<sup>a</sup>             | 1500                  | 8217                  |
| HPMC 6cp / PVP K12     | 2.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 2.0 / 0.5              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 0.5              | 380<sup>b</sup>              | 351                   | 2301                  |
| HPMC 6cp / PVP K17     | 2.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 2.0 / 0.5              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 1.0              | N.D                           | N.D                    | N.D                    |
|                         | 1.0 / 0.5              | 507<sup>b</sup>              | 451                   | 7239                  |
| Polymer / Polymer / Surfactant | Concentration (%, w/v) | Particle size (z-average, nm) | Particle size (d50, nm) | Particle size (d90, nm) |
|-------------------------------|------------------------|------------------------------|------------------------|------------------------|
| HPMC 6cp / PVP K25           | 2.0 / 1.0              | N.D                          | N.D                    | N.D                    |
|                               | 2.0 / 0.5              | N.D                          | N.D                    | N.D                    |
|                               | 1.0 / 1.0              | N.D                          | N.D                    | N.D                    |
|                               | 1.0 / 0.5              | N.D                          | N.D                    | N.D                    |
| HPMC 6cp / PVP K30           | 2.0 / 1.0              | N.D                          | N.D                    | N.D                    |
|                               | 2.0 / 0.5              | N.D                          | N.D                    | N.D                    |
|                               | 1.0 / 1.0              | N.D                          | N.D                    | N.D                    |
|                               | 1.0 / 0.5              | N.D                          | N.D                    | N.D                    |
| HPMC 6cp / PVP K90           | 2.0 / 1.0              | N.D                          | N.D                    | N.D                    |
|                               | 2.0 / 0.5              | N.D                          | N.D                    | N.D                    |
|                               | 1.0 / 1.0              | N.D                          | N.D                    | N.D                    |
|                               | 1.0 / 0.5              | N.D                          | N.D                    | N.D                    |

aN.D indicates that data cannot be measured and there is no data. bPrecipitation occurs due to particle agglomeration.

Table S4. Particle size of resveratrol nanosuspensions prepared using various polymer/polymer/surfactant combinations.
Table S5. Particle size of resveratrol nanosuspensions prepared using various resveratrol concentrations in Transcutol HP and various ratios of solvent/antisolvent using PVP VA64/PVP K12/SLS (1.0%/0.5%/0.1%, w/v).

| Resveratrol concentration in Transcutol HP | Ratio of solvent/anti-solvent | Resveratrol in nanosuspension | Particle size (z-average, nm) | Particle size (d50, nm) | Particle size (d90, nm) |
|-------------------------------------------|------------------------------|------------------------------|-----------------------------|------------------------|------------------------|
| 100 mg/mL                                 | 1/9                          | 10 mg/mL                     | 140.3                       | 101.4                  | 1180.9                 |
| 100 mg/mL                                 | 1/19                         | 5 mg/mL                      | 44.7                        | 60.0                   | 165.6                  |
| 200 mg/mL                                 | 1/19                         | 10 mg/mL                     | 1293.5                      | 1792.1                 | 34516.2                |
| 200 mg/mL                                 | 1/39                         | 5 mg/mL                      | 225.7                       | 117.2                  | 2780.4                 |

Table S6. Updated risk assessment of resveratrol nanosuspension after preformulation and screening study.

| CQAs                        | Parameters         | Risk level | Justification                                                                                                                                 |
|-----------------------------|--------------------|------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| Resveratrol concentration   | Low                | In a preliminary experiment, the resveratrol concentration was fixed at 100 mg/mL to satisfy the define QTPP. Therefore, the risk level was reduced to low. |
| Stabilizer type             | Low                | Based on preliminary experiments, PVP VA64, PVP K12, and SLS were selected as stabilizers. Therefore, the risk level was reduced to low. |
| Solvent type                | Low                | Based on preliminary experiments, Transcutol® HP was selected as a solvent. Therefore, the risk level was reduced to low. |
| Ratio of solvent/anti-solvent| Low               | Based on preliminary experiments, the solvent/anti-solvent ratio was fixed at 1/19 to satisfy the define QTPP. Therefore, the risk level was reduced to low. |
| Mixing speed                | Medium             | In a preliminary experiment, a nanosuspension that satisfied the target values was prepared at a mixing speed of 750 rpm. However, the mixing speed can still affect particle size. Therefore, the risk level was reduced to medium. |
| Mixing time                 | Low                | In preliminary experiments, the effect of mixing time on the particle size distribution was insignificant. Therefore, the risk level was reduced to low. |
| Injection rate (solvent)    | Medium             | In a preliminary experiment, a nanosuspension that satisfied the target values was prepared at an injection rate of 1.0 mL/min. However, the injection rate can still affect the particle size. Therefore, the risk level was reduced to medium. |
| Temperature                 | Medium             | In a preliminary experiment, a nanosuspension that satisfied the target values was prepared at 25°C. However, the temperature can still affect the particle size. Therefore, the risk level was reduced to medium. |
In preliminary experiments, PVP VA64, PVP K12, and SLS were selected as stabilizers. Therefore, the risk level was reduced to low.

Depending on the concentration of stabilizer, the surface charge of the particles is different. Therefore, the risk level was high.

In preliminary experiments, the solvent/anti-solvent ratio was fixed at 1/19 to satisfy the define QTPP. Therefore, the risk level was reduced to low.

### Table S7. Summary of results of regression analysis for the fitted model of the full factorial design.

| Response | $R^2$ | Pred. $R^2$ | PRESS | %CV | p-value | Remark |
|----------|-------|-------------|-------|-----|---------|--------|
| $Y_1$    | 0.9901| 0.4876      | 58.36 | 1.50| 0.0295  | Significant |
| $Y_2$    | 0.9912| 0.9271      | 7.70  | 0.41| 0.0028  | Significant |
| $Y_3$    | 0.7011| 0.1557      | 47.78 | 1.18| 0.0882  | Not significant |
| $Y_4$    | 0.4176| -0.5642     | 73.12 | 1.44| 0.4009  | Not significant |
| $Y_5$    | 0.3285| -0.0400     | 1166.64| 5.90| 0.1067  | Not significant |
| $Y_6$    | 0.9959| 0.9602      | 7.60  | 0.26| 0.0122  | Significant |
| $Y_7$    | 0.3503| -0.1103     | 517.28| 2.55| 0.0932  | Not significant |
| $Y_8$    | 0.7269| 0.4196      | 168.17| 1.36| 0.0204  | Significant |
| $Y_9$    | 0.9496| 0.7110      | 10.73 | 1.86| 0.0074  | Significant |

Regression equation of the fitted model

- $Y_1 = 49.97 + 0.97X_1 - 2.12X_2 - 0.93X_3 - 0.92X_1X_2 - 1.12X_1X_3 - 2.39X_2X_3$
- $Y_2 = 134.54 - 0.10X_1 - 2.28X_2 - 0.16X_3 - 1.85X_1X_2 - 2.10X_2X_3$
- $Y_3 = 155.63 - 0.03X_1 - 0.72X_2 - 2.11X_3X_2$
- $Y_4 = 162.25 + 0.17X_1 - 0.15X_2 - 1.55X_3X_2$
- $Y_5 = 175.88 + 6.79X_1$
- $Y_6 = 241.87 + 1.50X_1 + 0.71X_2 - 1.96X_3 - 0.85X_1X_2 + 4.05X_1X_3 + 0.30X_2X_3$
- $Y_7 = 258.09 - 4.52X_3$
- $Y_8 = 266.96 + 4.74X_2 + 1.97X_3$
- $Y_9 = -36.74 - 0.90X_1 - 0.10X_2 - 0.22X_3 + 1.88X_2X_3$

$R^2$, coefficient of determination; PRESS, predicted residual error sum of squares; CV, coefficient of variation.
### Table S8. Predicted values (95% prediction interval) for responses (Y$_1$–Y$_9$).

| Response | 95% PI (low) | 95% PI (high) | Predicted mean | Actual mean |
|----------|--------------|---------------|----------------|-------------|
| Y$_1$    | 35.4         | 58.1          | 46.7           | 46.3        |
| Y$_2$    | 134.3        | 149.3         | 141.8          | 139.2       |
| Y$_3$    | 149.0        | 169.6         | 159.3          | 154.6       |
| Y$_4$    | 167.5        | 183.7         | 175.6          | 169.7       |
| Y$_5$    | 147.6        | 169.4         | 158.5          | 157.7       |
| Y$_6$    | 241.1        | 260.0         | 250.5          | 255.0       |
| Y$_7$    | 234.2        | 281.6         | 257.9          | 252.9       |
| Y$_8$    | 240.3        | 286.8         | 263.5          | 250.9       |
| Y$_9$    | -43.37       | -35.45        | -39.41         | -38.02      |

PI, prediction interval.

### Table S9. Updated risk assessment of resveratrol nanosuspension after optimization study.

| CQAs                        | Parameters                        | Risk level | Justification                                                                                                                                 |
|-----------------------------|-----------------------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| Particle size               | (z-average, d$_{90}$)             |            | based on a preliminary experiment, the resveratrol concentration was fixed at 100 mg/mL. Therefore, the risk level was reduced to low.          |
| Resveratrol concentration   | Low                               |            | In preliminary experiments, PVP VA64, PVP K12, and SLS were selected as stabilizers. Therefore, the risk level was reduced to low.             |
| Stabilizer type             | Low                               |            | the particle size distribution in the optimized nanosuspension satisfies the set target range. Therefore, the risk level was reduced to low.  |
| Stabilizer concentration    | Low                               |            | Based on preliminary experiments, Transcutol® HP was selected as a solvent. Therefore, the risk level was reduced to low.                    |
| Solvent type                | Low                               |            | The particle size distribution of the nanosuspension prepared at 500 rpm–1000 rpm was within the set target range. Therefore, the risk level was reduced to low. |
| Ratio of solvent/anti-solvent | Low                              |            | Based on preliminary experiments, the ratio of solvent/anti-solvent was fixed at 1/19. Therefore, the risk level was reduced to low.           |
| Mixing speed                | Low                               |            | The particle size distribution of the nanosuspension prepared at 500 rpm–1000 rpm was within the set target range. Therefore, the risk level was reduced to low. |
| Mixing time                 | Low                               |            | Based on preliminary experiments, the effect of mixing time on the particle size distribution was insignificant. Therefore, the risk level was reduced to low. |
| Injection rate (solvent)    | Low                               |            | The particle size distribution of the nanosuspension prepared at an injection rate of 1.0 mL/min was within the set target range. Therefore, the risk level was reduced to low. |
| Parameter                      | Risk Level | Description                                                                                                                                                                                                 |
|-------------------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Temperature                   | Low        | The particle size distribution of the nanosuspension prepared at 20°C - 30°C was within the set target range. Therefore, the risk level was reduced to low.                                                     |
| Resveratrol concentration     | Low        | The effect of changes in the resveratrol concentration on the zeta potential is insignificant. Therefore, the risk level was low.                                                                              |
| Stabilizer type               | Low        | Based on preliminary experiments, PVP VA64, PVP K12, and SLS were selected as stabilizers. Therefore, the risk level was reduced to low.                                                                     |
| Stabilizer concentration      | Low        | In the optimized nanosuspension, the zeta potential value was -32.9 mV to -39.6 mV and satisfied the target range. Therefore, the risk level was reduced to low.                                               |
| Solvent type                  | Low        | The influence of the type of solvent on the surface charge of the particles was insignificant. Therefore, the risk was low.                                                                                     |
| Ratio of solvent/anti-solvent | Low        | Based on preliminary experiments, the solvent/anti-solvent ratio was fixed at 1/19. Therefore, the risk was reduced to low.                                                                                   |
| Mixing speed                  | Low        | The effect of mixing speed on the zeta potential was insignificant. Therefore, the risk was low.                                                                                                           |
| Mixing time                   | Low        | The effect of mixing time on the zeta potential was insignificant. Therefore, the risk was low.                                                                                                          |
| Injection rate (solvent)      | Low        | The effect of injection rate on the zeta potential was insignificant. Therefore, the risk was low.                                                                                                           |
| Temperature                   | Low        | The effect of temperature on the zeta potential was insignificant. Therefore, the risk was low.                                                                                                           |
| Resveratrol concentration     | Low        | Resveratrol is chemically stable when light is blocked. Nanosuspensions were prepared in a space where light is blocked, and the possibility of drug loss during the manufacturing process is insignificant. Therefore, the risk level was reduced to low. |
| Stabilizer type               | Low        |                                                                                                                                                                                                            |
| Stabilizer concentration      | Low        |                                                                                                                                                                                                            |
| Solvent type                  | Low        |                                                                                                                                                                                                            |
| Ratio of solvent/anti-solvent | Low        |                                                                                                                                                                                                            |
| Mixing speed                  | Low        |                                                                                                                                                                                                            |
| Mixing time                   | Low        |                                                                                                                                                                                                            |
| Injection rate (solvent)      | Low        |                                                                                                                                                                                                            |
| Temperature                   | Low        |                                                                                                                                                                                                            |
Table S10. Summary of long-term stability test results for the optimized resveratrol nanosuspension.

| Days | Particle size (z-average, nm) | Particle size (d90, nm) | Zeta potential (mV) | Drug content (%) |
|------|-----------------------------|------------------------|---------------------|------------------|
| 0    | 46.3                        | 157.7                  | −38.02              | 100.02           |
| 1    | 139.2                       | 255.0                  | N.D\(^a\)           | N.D\(^a\)        |
| 3    | 154.6                       | 252.9                  | N.D\(^a\)           | N.D              |
| 7    | 169.7                       | 250.9                  | N.D\(^a\)           | N.D              |
| 30   | 178.7                       | 302.9                  | −37.61              | 99.62            |
| 60   | 189.8                       | 311.3                  | N.D\(^a\)           | 99.21            |
| 90   | 197.9                       | 312.1                  | N.D\(^a\)           | 98.87            |
| 120  | 204.4                       | 311.1                  | N.D\(^a\)           | 98.55            |
| 150  | 209.4                       | 320.7                  | N.D\(^a\)           | 98.14            |
| 180  | 212.6                       | 321.0                  | −37.29              | 97.81            |

\(^a\)N.D indicates that data cannot be measured and there is no data.