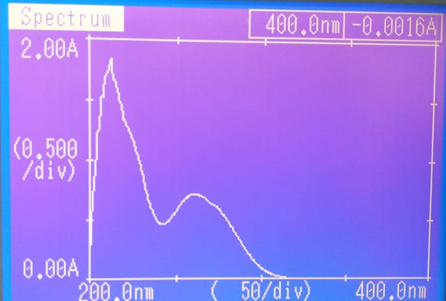
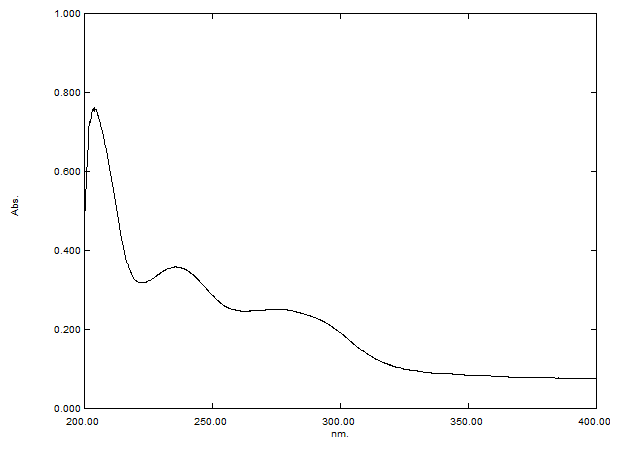
**Supplementary data**

**Fig. S1** **Absorption maxima (λmax) of SFN (A) and TAM (B)**

**Fig. S2 Standard calibration curve of TAM (A) and SFN (B)**

**Fig. S3 Response factor of TAM (A) and SFN (B)**

**Table S1 Observed Responses in Box-Behnken Trial Runs for TAM-SFN-Loaded NLCs**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **No. of runs** | **Methanol concentration (%)** | **pH of aqueous mobile phase** | **Flow rate (mL/min)** | **Y1** | **Y2** | **Y3** | **Y4** | **Y5** | **Y6** |
| 1 | -1 | -1 | 0 | 9.23 | 1.39 | 5723 | 5.9 | 1.41 | 6464 |
| 2 | -1 | 0 | 1 | 9.9 | 1.58 | 5723 | 7.32 | 1.76 | 6484 |
| 3 | 0 | 0 | 0 | 7.6 | 1.52 | 5782 | 4.13 | 1.78 | 6543 |
| 4 | -1 | 1 | 0 | 9.51 | 1.91 | 6028 | 6.12 | 2.2 | 6770 |
| 5 | 1 | -1 | 0 | 6.93 | 1.33 | 5661 | 3.22 | 1.59 | 6442 |
| 6 | 1 | 0 | -1 | 7.62 | 1.51 | 5770 | 4.45 | 1.69 | 6531 |
| 7 | 1 | 1 | 0 | 6.91 | 1.84 | 6023 | 3.75 | 2.02 | 6801 |
| 8 | 0 | 1 | 1 | 8.3 | 1.83 | 5991 | 4.83 | 2.15 | 6752 |
| 9 | 0 | 0 | 0 | 7.6 | 1.48 | 5730 | 4.13 | 1.76 | 6543 |
| 10 | 0 | 0 | 0 | 7.63 | 1.57 | 5790 | 4.16 | 1.78 | 6551 |
| 11 | -1 | 0 | -1 | 10.01 | 1.48 | 5760 | 6.36 | 1.66 | 6551 |
| 12 | 0 | 0 | 0 | 7.59 | 1.53 | 5772 | 4.13 | 1.79 | 6531 |
| 13 | 0 | -1 | -1 | 7.57 | 1.33 | 5664 | 4.1 | 1.62 | 6425 |
| 14 | 0 | 1 | -1 | 8.41 | 1.89 | 6021 | 4.94 | 2.17 | 6782 |
| 15 | 0 | 0 | 0 | 7.6 | 1.45 | 5782 | 4.13 | 1.78 | 6543 |
| 16 | 1 | 0 | 1 | 8.41 | 1.56 | 5816 | 4.59 | 1.71 | 6583 |
| 17 | 0 | -1 | 1 | 8.68 | 1.46 | 5670 | 5.21 | 1.66 | 6431 |

**Table S2 Coded independent with their lower and higher limit**

|  |  |  |  |
| --- | --- | --- | --- |
| **Factor Level used** | **Actual (coded)** | | |
| **Independent variable** | **Low (-1)** | **Medium (0)** | **High (+1)** |
| **X1= Methanol concentration (%)** | **60** | **70** | **80** |
| **X2= pH of aqueous mobile phase** | **3** | **3.5** | **4** |
| **X3= Flow rate (mL/min)** | **0.7** | **0.8** | **0.9** |

**Table S3 Correlation coefficient (r2) of dependent variables**

|  |  |  |  |
| --- | --- | --- | --- |
| **Dependent variable** | **r2** | **Actual r2** | **Predicted r2** |
| **RT (TAM)** | 0.9901 | 0.9774 | 0.8428 |
| **Tailing factor (TAM)** | 0.9765 | 0.9462 | 0.8465 |
| **Theoretical plates (TAM)** | 0.9769 | 0.9473 | 0.7567 |
| **RT (SFN)** | 0.9982 | 0.9958 | 0.9714 |
| **Tailing factor (SFN)** | 0.9797 | 0.9536 | 0.9131 |
| **Theoretical plates (SFN)** | 0.9966 | 0.9923 | 0.9580 |

**Table S4 (A) Robustness data of the proposed HPLC method of TAM**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameters** | **Variation** | **%RSD (Mean peak area)** | **Theoretical plates** | **Tailing factor** | **Retention time** | **%RSD assay** |
| **Wavelength (nm)** | 273 | 0.645 | 5781.55 | 1.52 | 7.59 | 0.894 |
| 275 | 0.512 | 5784.7 | 1.54 | 7.6 | 0.943 |
| 277 | 0.678 | 5782.51 | 1.53 | 7.61 | 1.23 |
| **Column Temperature (℃)** | 25 | 0..675 | 5783.3 | 1.52 | 7.58 | 0.76 |
| 30 | 0.986 | 5782.12 | 1.50 | 7.61 | 0.345 |
| 35 | 0.541 | 5788.86 | 1.51 | 7.62 | 0.95 |
| **Injection volume (µL)** | 15 | 0.865 | 5780.0 | 1.54 | 7.6 | 1.45 |
| 20 | 0.796 | 5778.76 | 1.53 | 7.58 | 0.56 |
| 25 | 0.675 | 5779.9 | 1.52 | 7.59 | 0.65 |

**Table S4 (B) Robustness data of the proposed HPLC method of SFN**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Parameter** | **Variation** | **%RSD (Mean peak area)** | **Theoretical plates** | **Tailing factor** | **Retention time** | **%RSD assay** |
| **Wavelength (nm)** | 207 | 0.764 | 6551.43 | 1.78 | 4.13 | 1.21 |
| 209 | 0.562 | 6543.7 | 1.79 | 4.12 | 0.94 |
| 211 | 0.342 | 6547.8 | 1.72 | 4.11 | 0.87 |
| **Column Temperature (℃)** | 25 | 0.576 | 6554.21 | 1.79 | 4.13 | 0.32 |
| 30 | 0.984 | 6544.04 | 1.76 | 4.14 | 0.76 |
| 35 | 0.567 | 6565.55 | 1.78 | 4.16 | 1.10 |
| **Injection volume (µL)** | 15 | 0.594 | 6543.5 | 1,76 | 4.11 | 0.74 |
| 20 | 0.872 | 6541.9 | 1,74 | 4.12 | 0.54 |
| 25 | 0.342 | 6554.03 | 1.73 | 4.11 | 0.38 |

**Table S5 (A) Intra-day precision of SFN**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level** | **Day 1** | **Day 2** | **Day 3** | **Mean** | **%RSD** |
| **LQC** | | | | | |
| **Retention time** | 4.13 | 4.14 | 4.12 | 4.13 | - |
| **Peak area** | 493456 | 495432 | 496784 | 495224 | 0.34 |
| **Theoretical plates** | 3245 | 3289 | 3251 | 3261.67 | - |
| **Tailing factor** | 1.78 | 1.76 | 1.79 | 1.78 | - |
| **% Recovery** | 99.70 | 100.30 | 101.50 | 100.50 | 0.91 |
| **MQC** | | | | | |
| **Retention time** | 4.13 | 4.14 | 4.13 | 4.13 | - |
| **Peak area** | 841456 | 841654 | 841987 | 841699 | 0.03 |
| **Theoretical plates** | 6554 | 6553 | 6557 | 6554.67 | - |
| **Tailing factor** | 1.76 | 1.79 | 1.76 | 1.77 | - |
| **% Recovery** | 100.10 | 99.70 | 100.60 | 100.13 | 0.45 |
| **HQC** | | | | | |
| **Retention time** | 4.15 | 4.13 | 4.11 | 4.13 | - |
| **Peak area** | 1151556 | 1143214 | 1159685 | 1151485 | 0.72 |
| **Theoretical plates** | 10106 | 10124 | 10185 | 10138.33 | - |
| **Tailing factor** | 1.75 | 1.75 | 1.78 | 1.76 | - |
| **% Recovery** | 98.60 | 101.20 | 100.40 | 100.07 | 1.33 |

**Table S5 (B) Intra-day precision of TAM**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level** | **Day 1** | **Day 2** | **Day 3** | **Mean** | **%RSD** |
| **LQC** | | | | | |
| **Retention time** | 7.59 | 7.61 | 7.62 | 7.61 | - |
| **Peak area** | 380993 | 381235.00 | 382374.00 | 381534.00 | 0.19 |
| **Theoretical plates** | 2997 | 2924 | 2961 | 2960.67 | - |
| **Tailing factor** | 1.54 | 1.51 | 1.53 | 1.53 | - |
| **% Recovery** | 100.40 | 98.70 | 101.20 | 100.10 | 1.28 |
| **MQC** | | | | | |
| **Retention time** | 7.62 | 7.58 | 7.59 | 7.60 | - |
| **Peak area** | 622277 | 629853.00 | 623489.00 | 625206.33 | 0.65 |
| **Theoretical plates** | 5782.00 | 5782.00 | 5781.00 | 5781.67 | - |
| **Tailing factor** | 1.52 | 1.49 | 1.54 | 1.52 | - |
| **% Recovery** | 99.80 | 103.40 | 101.20 | 101.47 | 1.79 |
| **HQC** | | | | | |
| **Retention time** | 7.61 | 7.59 | 7.58 | 7.59 | - |
| **Peak area** | 844554 | 849673.00 | 841902.00 | 845376.33 | 0.47 |
| **Theoretical plates** | 9789 | 9784 | 9786 | 9786.33 | - |
| **Tailing factor** | 1.55 | 1.51 | 1.55 | 1.54 | - |
| **% Recovery** | 102.70 | 99.81 | 103.20 | 101.90 | 1.80 |

**Table S5 (C) Inter-day precision of SFN**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level** | **Retention time** | **Peak area** | **Theoretical plates** | **Tailing factor** | **% Recovery** |
| **LQC** | | | | | |
| **Rep 1** | 4.13 | 499456 | 3268 | 1.78 | 100.2 |
| **Rep 2** | 4.14 | 497367 | 3291 | 1.77 | 98.99 |
| **Rep 3** | 4.13 | 498761 | 3256 | 1.76 | 99.99 |
| **Mean** | 4.13 | 498528 | 3271.67 | 1.77 | 99.72 |
| **% RSD** | - | 0.213 | - | - | 0.648 |
| **MQC** | | | | | |
| **Rep 1** | 4.14 | 841456 | 6553 | 1.76 | 98.65 |
| **Rep 2** | 4.13 | 841765 | 6548 | 1.79 | 100.34 |
| **Rep 3** | 4.13 | 845905 | 6543 | 1.75 | 102.10 |
| **Mean** | 4.13 | 843042 | 6548 | 1.77 | 100.36 |
| **% RSD** | - | 0.29 | - | - | 1.72 |
| **HQC** | | | | | |
| **Rep 1** | 4.13 | 1151556 | 10654 | 1.76 | 100.23 |
| **Rep 2** | 4.14 | 1152421 | 10983 | 1.78 | 98.70 |
| **Rep 3** | 4.13 | 1145267 | 10845 | 1.79 | 99.76 |
| **Mean** | 4.13 | 1149748 | 10827.33 | 1.78 | 99.56 |
| **% RSD** | - | 0.34 | - | - | 0.79 |

**Table S5 (D) Inter-day precision of TAM**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Level** | **Retention time** | **Peak area** | **Theoretical plates** | **Tailing factor** | **% Recovery** |
| **LQC** | | | | | |
| **Rep 1** | 7.61 | 380993.00 | 2783 | 1.54 | 100.90 |
| **Rep 2** | 7.63 | 389032.00 | 2789 | 1.55 | 99.80 |
| **Rep 3** | 7.58 | 385456.00 | 2698 | 1.59 | 101.20 |
| **Mean** | 7.61 | 385160.33 | 2756.67 | 1.56 | 100.63 |
| **% RSD** | - | 1.05 | - | - | 0.73 |
| **MQC** | | | | | |
| **Rep 1** | 7.59 | 622277.00 | 5745.00 | 1.55 | 99.80 |
| **Rep 2** | 7.58 | 623456.00 | 5786.00 | 1.59 | 101.20 |
| **Rep 3** | 7.62 | 627863.00 | 5789.00 | 1.54 | 102.50 |
| **Mean** | 7.60 | 624532.00 | 5773.33 | 1.56 | 101.17 |
| **% RSD** | - | 0.47 | - | - | 1.33 |
| **HQC** | | | | | |
| **Rep 1** | 7.59 | 844554.00 | 9754 | 1.55 | 100.70 |
| **Rep 2** | 7.60 | 845632.00 | 9775 | 1.54 | 101.20 |
| **Rep 3** | 7.62 | 849876.00 | 9789 | 1.59 | 99.90 |
| **Mean** | 7.60 | 846687.33 | 9772.67 | 1.56 | 100.60 |
| **% RSD** | - | 0.33 | - | - | 0.65 |

**Table S6 (A) Ruggedness data of TAM and SFN in different HPLC instrument with same analyst**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Drug** | **Level** | **Theoretical concentration** | **Recovered concentration** | | **% Recovery** | |
| **HPLC-1** | **HPLC-2** | **HPLC-1** | **HPLC-2** |
| **TAM** | LQC | 10 | 9.87±1.3 | 10.34±0.45 | 98.7 | 103.4 |
| MQC | 15 | 14.99±0.12 | 14.77±0.87 | 99.9 | 98.5 |
| HQC | 20 | 20.54±0.45 | 20.12±0.88 | 102.7 | 100.6 |
| **SFN** | LQC | 10 | 10.03±0.98 | 10.34±1.4 | 100.3 | 103.4 |
| MQC | 15 | 15.42±1.2 | 15.33±0.97 | 102.8 | 102.2 |
| HQC | 20 | 19.67±0.45 | 20.45±1.44 | 98.4 | 102.3 |

**Table S6 (B) Ruggedness data of TAM and SFN by different analyst in same HPLC instrument**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Drug | Level | Theoretical concentration | Recovered concentration | | % Recovery | |
| Analyst-1 | Analyst -2 | Analyst -1 | Analyst -2 |
| TAM | LQC | 10 | 10.45±1.5 | 9.89±0.64 | 104.5 | 98.9 |
| MQC | 15 | 14.65±1.2 | 15.41±0.34 | 97.7 | 102.7 |
| HQC | 20 | 19.57±0.78 | 20.52±0.59 | 97.9 | 102.6 |
| SFN | LQC | 10 | 10.53±0.55 | 9.56±1.23 | 105.3 | 95.6 |
| MQC | 15 | 14.982±0.76 | 15.09±1.4 | 99.9 | 100.6 |
| HQC | 20 | 20.45±1.54 | 20.16±0.89 | 102.3 | 100.8 |