Supporting Information for:

Loading of Coal Tar in Polymeric Nanoparticles as a Potential Therapeutic Modality for Psoriasis

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Figure S1. (A) Photographs of freshly prepared formulations of CT NPs (F1, F2, and F3) and empty PLGA NPs (F4); (B) Photograph of the failed formulation (F3).
HPLC method validation

The analytical method for CT was validated according to the “Guidance for Industry, Bioanalytical Method Validation, FDA” and International Conference on Harmonization (ICH) guidelines using quality control (QC) samples. To ensure and evaluate the method's validity and integrity, the linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), stability, selectivity, and robustness of the method were evaluated. Three QC samples were prepared from a CT standard stock solution in ACN (1 mg/mL) and were categorized as Low (30 μg/mL), Mid (250 μg/mL), and High (1000 μg/mL) QC.

Linearity

Linearity was determined by constructing calibration curves using eight concentrations: 10, 50, 100, 250, 500, 750, 1000, and 1250 μg/mL. The calibration curves were created by plotting the peak areas of the freshly prepared samples versus concentration, and the regression analysis was performed using Microsoft Excel. A correlation coefficient ($R^2$) of 0.999 was acknowledged as evidence of an acceptable fit of the data to the regression line.

Intra-day accuracy and precision

Six replicate injections of three different concentrations (30, 250 and 1000 μg/mL) for CT were used to evaluate the intra-day accuracy and precision. The concentrations were calculated using the regression equation of the calibration curve. The mean deviation from the actual value was accepted as the measure of accuracy, which was calculated according to Equation (1):

\[
\text{Accuracy} \% = 100 - \frac{(\text{True concentration} - \text{mean of measured concentrations})}{\text{True concentration}} \times 100\%
\]  

Equation (1)

Precision was calculated according to Equation (2):
\[ CV \% = \frac{SD \text{ of measured concentrations}}{Mean \text{ of measured concentrations}} \times 100\% \] (2)

**Inter-day accuracy and precision**

QC samples for CT were used over three days to evaluate the inter-day accuracy and precision. The concentrations were calculated by applying the calibration curve's regression equation. The mean deviation from the actual value served as the measure of the accuracy. Accuracy and precision were calculated according to Equation (1) and Equation (2), respectively.

**LOD and LOQ**

LOQ was determined based on the SD of response and slope where the SD of y-intercepts of regression lines were used as the standard deviation according to ICH guidelines. The LOD and LOQ were calculated using Equation (3) and Equation (4), respectively:

\[ LOD = \frac{3.3 \times SD}{\text{Slope}} \] (3)

\[ LOQ = \frac{10 \times SD}{\text{Slope}} \] (4)

**Stability**

Benchtop stability was studied by measuring QC samples' recovery over 6, 12, 24, and 48 h. The recovery of the samples was calculated using Equation (5):

\[ \text{Stability} \% = 100 - \frac{\text{Mean at initial time} - \text{Mean after a specific time}}{\text{Mean at initial time}} \] (5)

**Selectivity**

The method selectivity was determined by screening six different injections of the blank, PLGA, PLGA NPs, PLGA-CT mixture, and CT NPs to ensure no interference between the analyte (CT) and other ingredients.
**Robustness**

The effect of small variations in flow rate (± 5%) and column temperature (± 5°C) was assessed in terms of number of theoretical plates and asymmetry. The capacity factor ($K'$), number of theoretical plates ($N$), and asymmetry ($As$) were calculated using Equation (6), Equation (7), and Equation (8), respectively:

$$K' = \frac{t_r-t_0}{t_0}$$  \hspace{1cm} (6)

$$N = \frac{16}{\left(W_b\right)^2}$$  \hspace{1cm} (7)

$$As = \frac{B}{A}$$  \hspace{1cm} (8)

Where $t_r$ is the retention time of the analyte, $t_0$ is “the dead time” or the retention time of non-retained compounds, $w_b$ is the peak width at the base, $B$ is the distance between peak maximum and peak front, and $A$ is the distance between peak maximum and peak-end at 10% of peak height.
Validation results

Upon HPLC method development, CT could be detected at 270 nm using ACN as a mobile phase delivered at 0.5 mL/min. The CT peak appeared at 6.44 min, as illustrated in Figure S2.

![HPLC-UV chromatogram](image)

**Figure S2.** The HPLC-UV chromatogram of a sample containing CT using ACN delivered at a rate of 0.5 mL/min and detected at 270 nm.

**Linearity**

Calibration curves were established by injecting eight freshly prepared standard solutions of CT over the range of 10–1250 μg/mL and plotting peak areas versus CT concentration of each standard solution (Figure S3). The linearity parameters of six calibration curves for CT are listed in Table S1. The R² value for these curves were in the range 0.9974–0.9993, indicating that the method used was linear.
Figure S3. Representative CT standard calibration curve.

Table S1. Linearity parameters of CT calibration curves.

| Calibration curves | Concentration of CT (µg/mL) |   |   |   |   |   |   |   |   |   |
|--------------------|-----------------------------|---|---|---|---|---|---|---|---|---|
|                    | 10  | 50  | 100 | 250 | 500 | 750 | 1000 | 1250 |   | R² |
| 1 Peak area        | 91888 | 224119 | 466427 | 1242000 | 2394607 | 3488765 | 4691450 | 5672038 | 0.9993 |
| 2 Peak area        | 97686 | 243834 | 493491 | 1242862 | 2478780 | 3603773 | 4829683 | 5784358 | 0.9990 |
| 3 Peak area        | 96365 | 255419 | 489108 | 1235313 | 2424479 | 3508064 | 4572539 | 5568071 | 0.9989 |
| 4 Peak area        | 95304 | 261533 | 522571 | 1299718 | 2514564 | 3560007 | 4522547 | 5546518 | 0.9975 |
| 5 Peak area        | 96274 | 258739 | 491374 | 1227435 | 2658166 | 3760077 | 4976863 | 6079580 | 0.9989 |
| 6 Peak area        | 96172 | 270560 | 548894 | 1335942 | 2614810 | 4051265 | 5032802 | 6117442 | 0.9974 |
| Mean peak area     | 95615 | 252367 | 501978 | 1263878 | 2514234 | 3661992 | 4770981 | 5794668 | 0.9989 |

Accuracy and precision

As shown in Table S2. The method's intra-day accuracy ranged from 85.64 to 99.90%, and the intra-day precision ranged from 0.47 to 2.49%. In Table S3, the method's inter-day accuracy ranged from 88.96 to 103.50%, and the inter-day precision ranged from 2.72 to 4.73%. These
results indicate an accurate and precise method of analysis according to the guidelines, where CV% of inter and intra-day precision and the accuracy percentages did not exceed 15% over the three determined concentrations.

Table S2. Intra-day accuracy and precision for CT.

| Analyzed on day | QC Low (30 μg/mL) | QC Mid (250 μg/mL) | QC High (1000 μg/mL) |
|-----------------|-------------------|--------------------|----------------------|
| **Day 1**       |                   |                    |                      |
| 30.31           | 265.00            | 912.44             |
| 30.35           | 266.23            | 913.33             |
| 30.41           | 268.24            | 910.67             |
| 30.27           | 267.94            | 956.48             |
| 32.45           | 268.74            | 912.41             |
| 30.01           | 268.49            | 901.04             |
| **Mean concentration** | **30.63** | **267.44** | **917.73** |
| **Precision (CV%)** | **2.49** | **0.47** | **1.80** |
| **Accuracy (%)** | **102.12** | **106.98** | **91.77** |

Table S3. Inter-day accuracy and precision for CT.

| Day of analysis | Coal tar concentration |
|-----------------|------------------------|
|                 | QC Low (30 μg/mL)      | QC Mid (250 μg/mL) | QC High (1000 μg/mL) |
| **Day 1**       |                        |                    |                      |
|                 | 30.31                  | 265.00             | 912.44               |
|                 | 30.35                  | 266.23             | 913.33               |
|                 | 30.41                  | 268.24             | 910.67               |
|                 | 30.27                  | 267.94             | 956.48               |
|                 | 32.45                  | 268.74             | 912.41               |
|                 | 30.01                  | 268.49             | 901.04               |
| **Day 2**       |                        |                    |                      |
|                 | 27.39                  | 259.28             | 889.00               |
|                 | 27.42                  | 257.98             | 903.04               |
|                 | 27.42                  | 259.45             | 893.30               |
|                 | 27.29                  | 258.96             | 895.64               |
|                 | 27.39                  | 259.01             | 905.17               |
|                 | 27.70                  | 259.26             | 890.59               |
| **Day 3**       |                        |                    |                      |
|                 | 28.55                  | 249.66             | 865.50               |
|                 | 28.65                  | 249.20             | 855.82               |
|                 | 28.53                  | 250.19             | 862.36               |
|                 | 28.48                  | 249.91             | 851.51               |
|                 | 28.87                  | 250.05             | 846.08               |
|                 | 28.80                  | 250.12             | 847.91               |
| **Mean concentration** | **28.90** | **258.76** | **889.57** |
| **Precision (CV%)** | **4.73** | **2.72** | **3.12** |
| **Accuracy (%)** | **96.35** | **103.50** | **88.96** |
**LOD and LOQ**

After applying Equation (3) and Equation (4), the LOQ for CT was found to be 1.2 µg/mL, which was much lower than the lowest concentrations used throughout this study (Table S4).

**Table S4.** LOD and LOQ values using the SD and slope method.

| Parameter            | Result |
|----------------------|--------|
| LOD (µg/mL)          | 0.4    |
| LOQ (µg/mL)          | 1.2    |
| Peak Height (mAU)    | 10.0   |
| SD                   | 587.1  |
| Slope                | 4560.7 |
| Average of intercept | 53892  |

**Selectivity**

The maximum concentration of each of the additives used during sample preparation was analyzed and tested for any interference. The chromatograms in Figure S4 to Figure S8 show that there were no interfering peaks from these materials at the retention time of CT. Accordingly, the method was determined to be selective for the analyte.

**Figure S4.** The HPLC-UV chromatogram of a blank sample using ACN delivered at a rate of 0.5 mL/min and detected at 270 nm.
Figure S5. The HPLC-UV chromatogram of a sample containing PLGA dissolved in ACN delivered at a rate of 0.5 mL/min and detected at 270 nm.

Figure S6. The HPLC-UV chromatogram of a sample containing a physical mixture of CT and PLGA dissolved in ACN delivered at a rate of 0.5 mL/min and detected at 270 nm.

Figure S7. The HPLC-UV chromatogram of a sample containing blank PLGA NPs dissolved in ACN delivered at a rate of 0.5 mL/min and detected at 270 nm.
**Figure S8.** The HPLC-UV chromatogram of a sample containing CT-loaded PLGA NPs dissolved in ACN delivered at a rate of 0.5 mL/min and detected at 270 nm.

**Stability**

The results of the benchtop stability of CT samples are shown in Table S5 and indicate that the QC samples at the three different concentrations were all stable for 48 h at room temperature.

| QC sample          | Stability % |
|--------------------|-------------|
|                    | T₀ | T₆  | T₁₂ | T₂₄ | T₄₈         |
| Low QC (30 μg/mL)  | 100| 105.911| 100.1748| 97.72656| 99.1815846 |
| Mid QC (250 μg/ml) | 100| 100.6381| 101.563| 99.05587| 100.24489  |
| High QC (1000 μg/mL)| 100| 100.5171| 101.1568| 98.86149| 100.129452 |

**Robustness**

For the evaluation of robustness, six replicates of the high QC sample were tested for capacity factor (K'), number of theoretical plates (N), and asymmetry (As) at each variation. The results are shown in Table S6:

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Table S6. Results of robustness study.

| Parameter studied      | Average of 6 replicates | \( K' \) | \( N \) | \( As \) | \( Rt \) |
|------------------------|-------------------------|---------|-------|--------|-------|
| Nominal conditions     | 4575824                 | 1.40    | 2241  | 0.99   | 6.44  |
| Flow rate (0.45 mL/min)| 4629832                 | 1.40    | 2241  | 0.99   | 6.44  |
| Flow rate (0.55 mL/min)| 4647498                 | 1.40    | 2587  | 0.98   | 6.41  |
| Column 1               | 4523754                 | 1.50    | 2454  | 0.94   | 6.44  |
| Column 2               | 4626953                 | 1.50    | 2395  | 1.10   | 6.44  |
| Wavelength 275 nm      | 4661157                 | 1.40    | 2415  | 0.99   | 6.44  |
| Wavelength 265 nm      | 4663967                 | 1.40    | 2351  | 0.98   | 6.44  |
| Temperature 30 °C      | 4398172                 | 1.50    | 2495  | 1.10   | 6.44  |
| Temperature 20 °C      | 4462841                 | 1.40    | 2456  | 1.02   | 6.44  |

A higher \( K' \) value indicates that the sample is positively retained and firmly bound to the column's stationary phase. Values between 1 and 10 are considered acceptable. As demonstrated in Table S6, \( K' \) values were above 1 with the nominal condition and after slight variations were made, indicating good retention of the analyte as compared to the non-retained compound. The number of theoretical plates (\( N \)) measures the HPLC column's peak dispersion, reflecting the column performance. In general, \( N \) values should be more than 2000. The higher the \( N \) values, the narrower peaks and better resolution are obtained. \( N \) was more than 2000 for the nominal condition and throughout all the variations, indicating the excellent performance of the column. In an ideal situation, peaks should be symmetrical; however, due to various effects, peaks may often show a tailing behavior or a fronting peak shape, which presents a problem with resolution and quantitation of the peaks within a chromatogram. The accuracy of quantitation decreases with the increase in peak tailing because of the difficulties encountered in determining the peak end and the area under the peak. It is recommended to keep \( As \) values ≤ 1.2. This was achieved with the nominal condition and after variations were made to the method. Accordingly, the results indicated that the method was rigid and robust to small variations.