

SUPPLEMENTARY MATERIAL TO
Analytical method development and validation of antifungal drugs in updated ointment formulation using UV spectroscopy and RP-HPLC

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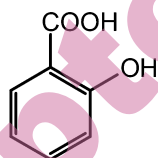


FIG S1. Salicylic acid

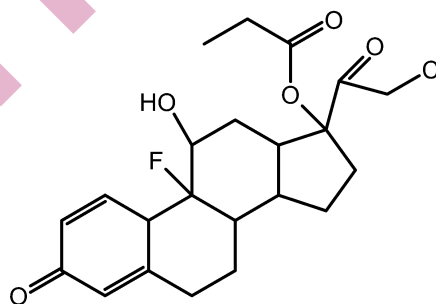


FIG S2. Clobetasol propionate

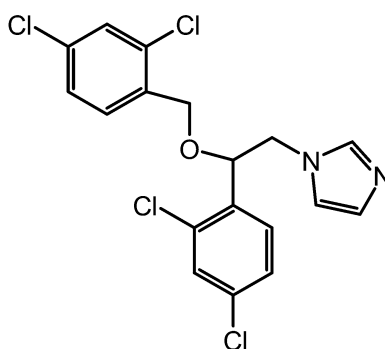


FIG S3. Miconazole nitrate

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Table SI. Data for formulation

| Ingredients | Quantity(g) |
|--------------------------|-------------|
| | TF 1 |
| Polyethylene glycol 400 | 35 |
| Polyethylene glycol 4000 | 15 |
| Clobetasol propionate | 0.015 |
| Miconazole nitrate | 0.6 |
| Salicylic acid | 0.9 |

Table SII. Data for evaluation parameters.

| Evaluation parameters | Formulation code | | |
|-----------------------|-----------------------|-----|-----|
| | TF 1 | | |
| Appearance | White | | |
| Homogeneity | Excellent | | |
| Consistency | Semisolid | | |
| pH | 4.9 ± 0.06 | | |
| Viscosity | 822000 | | |
| Spreadability | 17 cm ² /s | | |
| Drug content | CP | MN | SA |
| | 96% | 99% | 95% |

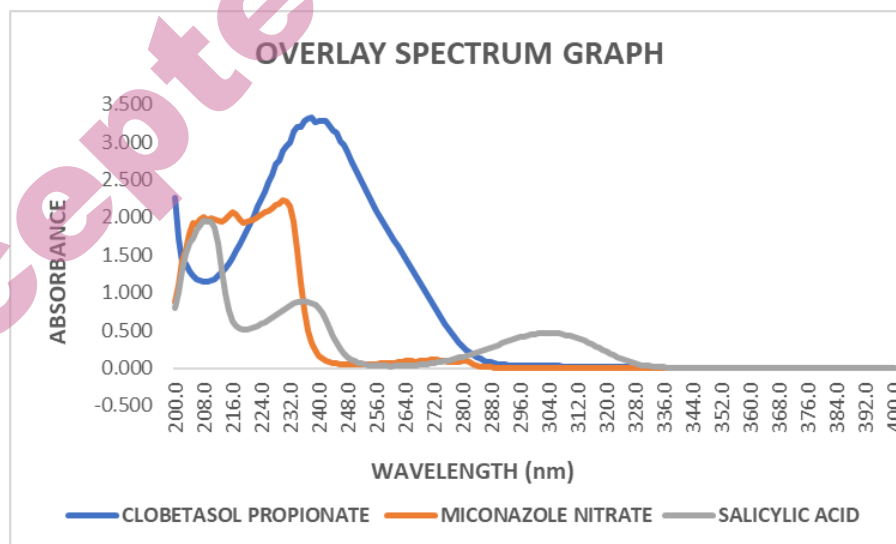
FIG S4. UV Spectrum overlay of clobetasol propionate (20 µg mL⁻¹), miconazole nitrate (20 µg mL⁻¹), salicylic acid (20 µg mL⁻¹).

Table SIII. Data for the amount of drug present in formulation using UV and MATLAB

| Drug | Amount taken ($\mu\text{g mL}^{-1}$) | Absorbance | Amount present ($\mu\text{g mL}^{-1}$) | % Assay |
|------|---|------------|---|---------|
| CP | 10 | 2.0115 | 9.86 | 98.6 |
| MN | 60 | 1.9838 | 55.32 | 92.2 |
| SA | 30 | 2.9853 | 32.911 | 109.70 |

Table SIV. Data for system suitability parameters

| Parameters | Observed results (mean \pm SD) (n=3) | | | % RSD | | |
|--------------------------------|---|-------------------------|--------------------------|--------|---------|--------|
| | CP | MN | SA | CP | MN | SA |
| Retention time (Rt) in mins | 5.128 \pm 0.0097 | 12.595 \pm 0.0244 | 2.539 \pm 0.0054 | 0.1893 | 0.1935 | 0.2163 |
| Area | 42912 \pm 14933.35 | 72337 \pm 27396.39 | 597798 \pm 214211.7 | 34.8 | 37.8732 | 35.833 |
| Theoretical plates (N) | 7,476 \pm 266.97 | 15,362 \pm 856.09 | 2871 \pm 158.32 | 3.5713 | 5.5729 | 5.5148 |
| Tailing factor (T) | 1.064 \pm 0.03724 | 1.092 \pm 0.0206 | 0.901 \pm 0.0451 | 3.4996 | 1.8924 | 5.0126 |
| Capacity factor (K') | 1.019 \pm 0.002571 | 3.962 \pm 0.0125 | - | 0.2522 | 0.3168 | - |
| Resolution | 12.127 \pm 0.22150 | 23.2 \pm 0.4675 | - | 1.8266 | 2.1014 | - |
| HETP | 20.086 \pm 0.7216 | 9.787 \pm 0.5101 | 52.374 \pm 2.8575 | 3.5927 | 5.2127 | 5.4559 |

Table SV. Linearity data for clobetasol propionate, miconazole nitrate, salicylic acid in RP-HPLC

| Clobetasol propionate concentration ($\mu\text{g mL}^{-1}$) | Clobetasol propionate peak area (n=3) | Miconazole nitrate concentration ($\mu\text{g mL}^{-1}$) | Miconazole nitrate peak area (n=3) | Salicylic acid concentratio n ($\mu\text{g mL}^{-1}$) | Salicylic acid peak area (n=3) |
|--|--|---|--|---|---|
| 5 | 25,500 | 30 | 40,085 | 15 | 3,31,393 |
| 7.5 | 33,022 | 45 | 54,898 | 22.5 | 4,52,794 |
| 10 | 40,568 | 60 | 68,046 | 30 | 5,92,080 |
| 12.5 | 52,929 | 75 | 89,774 | 37.5 | 7,58,835 |
| 15 | 62,539 | 90 | 1,08,881 | 45 | 8,53,890 |

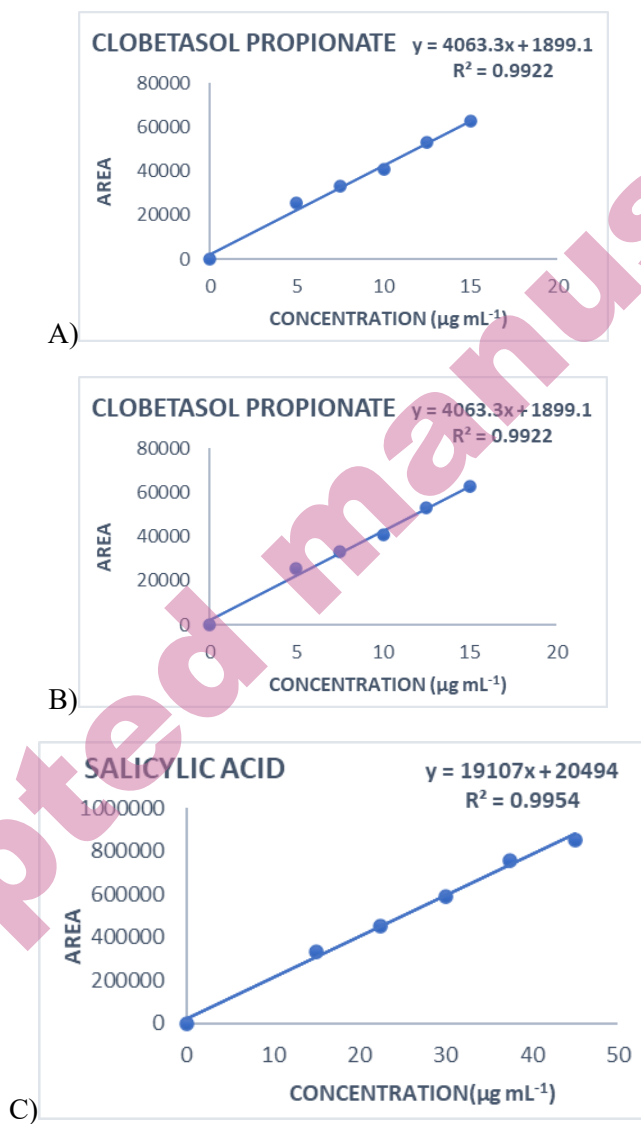


Fig S5. Data for linearity in RP-HPLC

Table SVI: Data for assay of ointment

| Drug | Amount taken ($\mu\text{g mL}^{-1}$) | Peak area (n=3) | Amount obtained ($\mu\text{g mL}^{-1}$) | Amount obtained | | | % Assay |
|------|--|-----------------|---|-----------------|--------|-------|---------|
| | | | | mean | SD | % RSD | |
| CP | 10 | 41614 | 9.65 | 9.58 | 0.0624 | 0.651 | 95.8 |
| | | 41132 | 9.53 | | | | |
| | | 41248 | 9.56 | | | | |
| MN | 60 | 72382 | 60.03 | 59.88 | 0.1450 | 0.242 | 99.8 |
| | | 72040 | 59.74 | | | | |
| | | 72199 | 59.88 | | | | |
| SA | 30 | 568558 | 28.38 | 28.36 | 0.0472 | 0.166 | 94.5 |
| | | 568915 | 28.4 | | | | |
| | | 567202 | 28.31 | | | | |

Table SVII. LOD and LOQ data for clobetasol propionate, miconazole nitrate, salicylic acid

| Drug | Clobetasol propionate ($\mu\text{g mL}^{-1}$) | Miconazole nitrate ($\mu\text{g mL}^{-1}$) | Salicylic acid ($\mu\text{g mL}^{-1}$) |
|------|---|--|--|
| LOD | 1.49 | 8.72 | 3.37 |
| LOQ | 4.53 | 26.43 | 10.22 |

Table SVIII. Robustness data for $\pm 0.1 \text{ mL min}^{-1}$ in flow rate

| parameters | Peak area (n=3) | | | Mean \pm SD | | | % RSD | | |
|---|-----------------|--------|--------|---------------|--------|--------|-------|-------|-------|
| | CP | MN | SA | CP | MN | SA | CP | MN | SA |
| Minus flow rate (1.1 mL min^{-1}) | 40,6 | 72,7 | 5,67,4 | 41,13 | 72,39 | 5,68,1 | 1.09 | 0.504 | 0.221 |
| | 20 | 70 | 80 | | | | | | |
| | 41,2 | 72,3 | 5,67,2 | | | | | | |
| | 93 | 82 | 78 | | | | | | |
| | 41,4 | 72,0 | 5,69,5 | | | | | | |
| Plus, the flow rate (1.3 mL min^{-1}) | 77 | 40 | 50 | 41,22 | 71,69 | 5,68,6 | 0.89 | 0.262 | 0.068 |
| | 41,6 | 71,5 | 5,68,2 | | | | | | |
| | 23 | 21 | 54 | | | | | | |
| | 40,8 | 71,8 | 5,69,0 | | | | | | |
| | 92 | 56 | 01 | | | | | | |
| 41,1 | 71,5 | 5,68,8 | 52 | 05 | 389.52 | 84 | 29 | 49 | |
| 54 | 41 | 19 | | | | | | | |

Table SIX: Robustness data for ± 2 °C temperature

| Parameters | Peak area (n=3) | | | Mean \pm SD | | | % RSD | | |
|------------------------|-----------------|--------|----------|------------------------|------------------------|---------------------------|--------|---------|---------|
| | CP | MN | SA | CP | MN | SA | CP | MN | SA |
| Minus temp. (28 °C) | 41,518 | 71,652 | 5,70,445 | 41,191 \pm 372.93 | 71,180 \pm 635.24 | 5,70,729 \pm 344.48 | 0.9054 | 0.89244 | 0.06036 |
| | 41,271 | 70,458 | 5,70,629 | | | | | | |
| | 40,785 | 71,431 | 5,71,112 | | | | | | |
| Plus, temp (32 °C) | 41,145 | 71,121 | 5,73,696 | 40,818 \pm 789.06 | 72,161 \pm 901.42 | 5,70,557 \pm 4506.48 | 1.9331 | 1.24919 | 0.78984 |
| | 39,918 | 72,644 | 5,72,581 | | | | | | |
| | 41,391 | 72,718 | 5,65,393 | | | | | | |

Table SX: Robustness data for ± 2 nm wavelength

| Parameters | Peak area (n=3) | | | mean \pm SD | | | % RSD | | |
|---------------------------------|-----------------|--------|----------|------------------------|-------------------------|----------------------------|--------|---------|---------|
| | CP | MN | SA | CP | MN | SA | CP | MN | SA |
| Minus wavelength (280 nm) | 41,518 | 71,841 | 5,68,753 | 41,361 \pm 136.69 | 72,070 \pm 207.811 | 5,69,060 \pm 779.258 | 0.3305 | 0.28835 | 0.13694 |
| | 41,293 | 72,247 | 5,68,481 | | | | | | |
| | 41,271 | 72,121 | 5,69,946 | | | | | | |
| Plus, wavelength (285 nm) | 40,892 | 71,950 | 5,75,985 | 41,100 \pm 456.08 | 72,160 \pm 216.25 | 5,77,079 \pm 1345.082 | 1.1097 | 0.29968 | 0.23308 |
| | 40,785 | 72,148 | 5,76,672 | | | | | | |
| | 41,623 | 72,382 | 5,78,581 | | | | | | |