

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

RETHINA KARUPPIAHYA¹, SUBA GEETHA ARUNACHALAM¹, SARAVANAN VENKATTAPURAM SAMPATH¹, SAMBATHKUMAR RAMANATHAN¹, ANANDA THANGADURAI SUBRAMANIAM², RAVIKUMAR RAMASAMY¹, ANUPRINCY PAULMURUGAN¹ and JAMBULINGAM MUNUSAMY^{1*}

¹Department of Pharmaceutical Analysis, The Erode College of Pharmacy, Erode, Tamil Nadu, India and ²Department of Pharmaceutical Analysis, JKKN College of Pharmacy, Kumarapalayam, Namakkal District, Tamil Nadu, India

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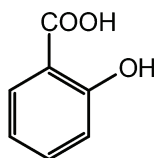


Fig. S-1. Salicylic acid

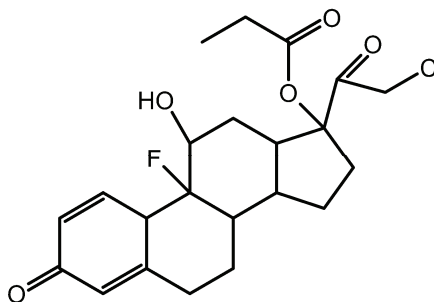


Fig. S-2. Clobetasol propionate

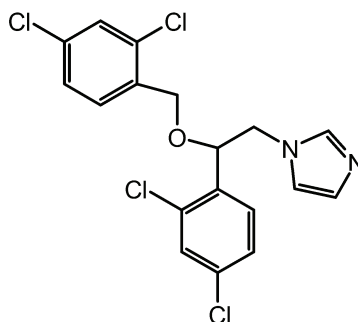


Fig. S-3. Miconazole nitrate

*Corresponding author. E-mail: jambulingam48@gmail.com

Table S-I. 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 S-II. 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 96%	MN 99%	SA 95%

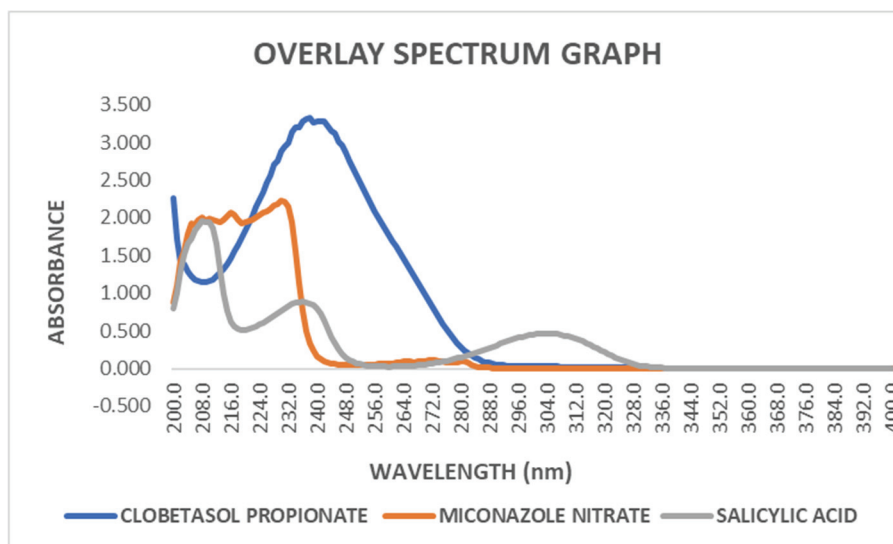
Fig. S-4. UV Spectrum overlay of clobetasol propionate (20 µg mL⁻¹), miconazole nitrate (20 µg mL⁻¹), salicylic acid (20 µg mL⁻¹).

Table S-III. 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 S-IV. 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 S-V. 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

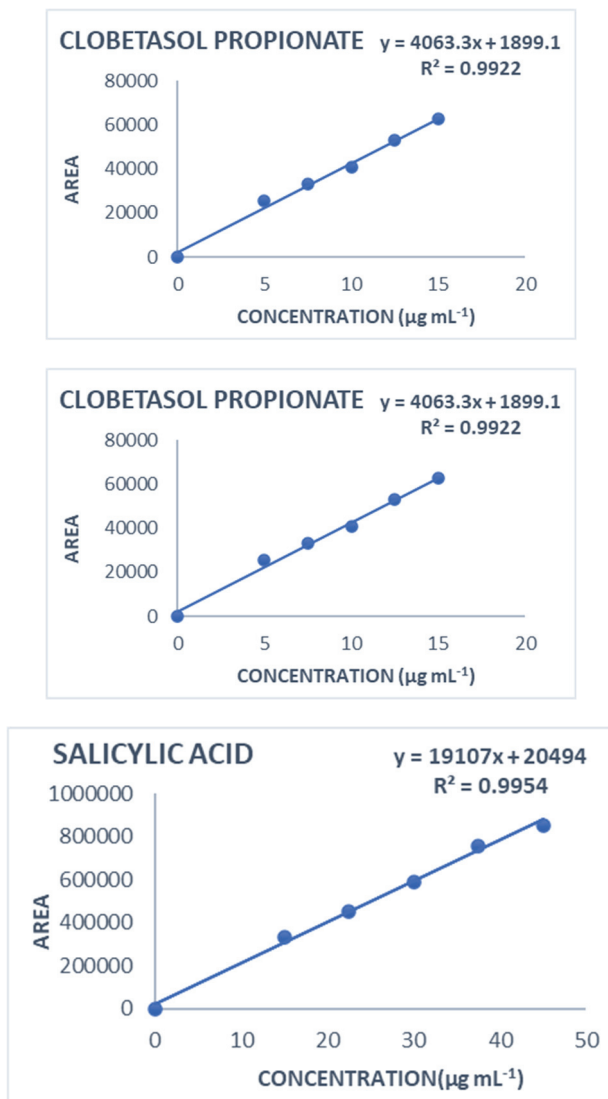


Fig. S-5. Data for linearity in RP-HPLC

Table S-VI. 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 S-VII. 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 S-VIII. 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	370.3	187.9	389.52	84	29	49	
54	41	19	52	05					

Table S-IX. 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 S-X. 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						