

Analysis of Clobetasol propionate in whitening cream

on Chromolith[®] HighResolution RP-18e 100 x 2 mm I.D. Column

Bleaching or skin whitening creams are widely used to reduce melanin in the skin. These consumer products require analytical quality control. This Application Note shows on the testing of clobetasol propionate in formulated cream products.

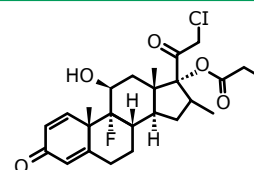
A Chromolith[®] HighResolution RP-18 endcapped column 100 x 2 mm I.D. was used with HPLC-UV detection.

Due to the very good permeability and the low backpressure of this column an analytical HPLC-System with a micro cell could be used. In addition, the rugged monolithic silica skeleton results in significant longer column life-time in comparison to testing methods using particulate columns.

Experimental

Chromatographic Conditions:

Column:	Chromolith [®] HighResolution RP-18e 100 x 2 mm [1.52322]
Mobile phase A:	Water
Mobile Phase B:	Methanol
Isocratic:	A/B 25/75 (v/v)
Flow Rate:	189 µL/min
Temperature:	25 °C
Pressure Drop:	53 bar (768 psi)
Injection volume:	1 µL Standard, 4 µL Sample
Diluent:	Methanol
Detection:	Ultimate 3000; at UV = 240 nm (micro flow cell; 1.4 µL/7 mm)



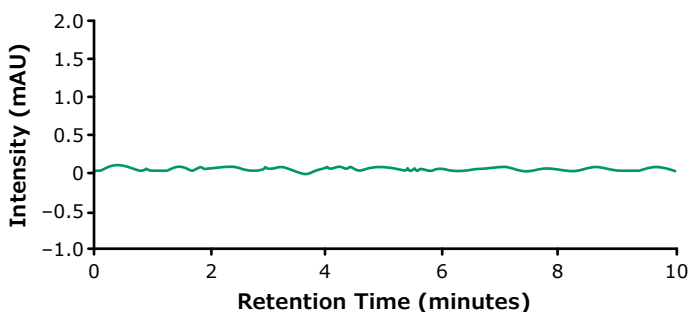
Clobetasol propionate
(21-Chloro-9-fluoro-11 β ,17-dihydroxy-16 β -methylpregna-1,4-diene-3,20-dione 17- propionate)

Sample:

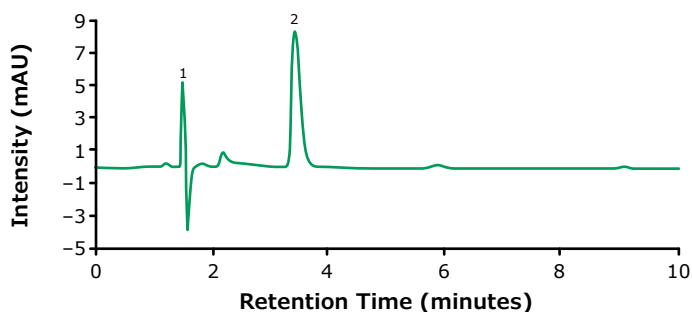
Standard solution (10 µL/mL):	Weigh accurately 5 mg of the standard into a 50 mL volumetric flask and add 40 mL diluent. Sonicate for 5 min and fill up to mark with diluent. Pipette 5 mL of this solution to a 50 mL volumetric flask and fill up to volume with diluent. Pass through 0.45 µm syringe filter before injection.
Sample solution:	Place 1 g of homogenized cream in a 50 mL volumetric flask, add 30 mL of water/methanol 30/70 (v/v) and sonicate for 5 minutes. Fill up to volume with water/methanol 30/70 (v/v). Transfer 3 mL of this suspension into a 25 mL volumetric flask and fill up to volume with diluent. Pass through 0.45 µm syringe filter before injection.

Application Note Clobetasol propionate

Blank Run

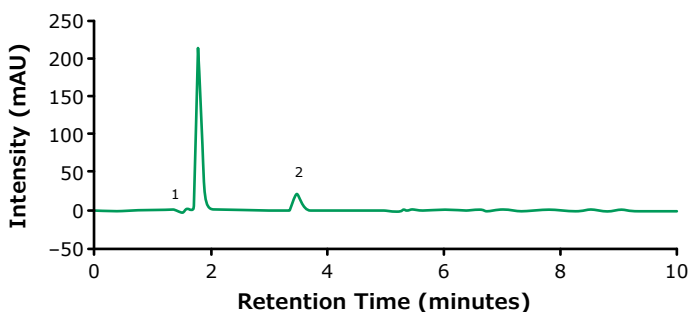


Standard Solution

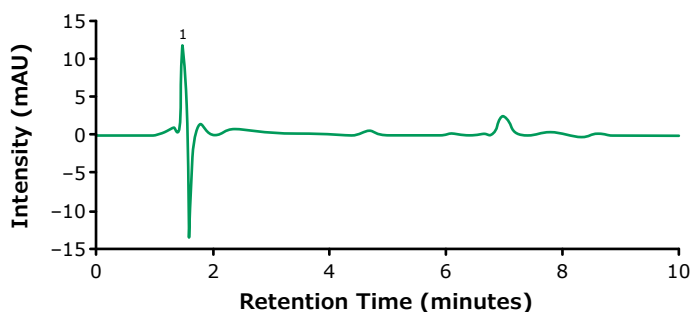


No.	Compound	Retention Time (min)	S/N	Area (mAU*min)	Tailing Factor
1	t ⁰ void volume	1.2			
2	Clobetasol propionate	3.4	140.1	1.3689	1.5

Cream with Clobetasol propionate



Cream without Clobetasol propionate



Chromatographic Data [Cream with Clobetasol propionate]

No.	Compound	Retention Time (min)	S/N	Area (mAU*min)	Tailing Factor
1	t ⁰ void volume	1.4			
2	Clobetasol propionat	3.5	380.8	3.3183	1.5

Results and Discussion

The determination of Clobetasol propionate in cream was achieved with a LOD less than 3 µg/mL and a LOQ less than 7 µg/mL using a Chromolith® HighResolution RP-18e 100 x 2 mm I.D. column. The calculated concentration of Clobetasol propionate in the sample [Cream with Clobetasol propionate] was 0.073 mg/g.

Specificity: Inject standard solution of Clobetasol propionate (10 µg/mL) and determine the retention time and content of desired analyte

No.	Compound	Retention Time (min)	Area (counts)	Tailing Factor	S/N
1	Clobetasol propionate	3.4	1.3941	1.5	140.1

Standard repeatability cream with Clobetasol propionate mAU*min

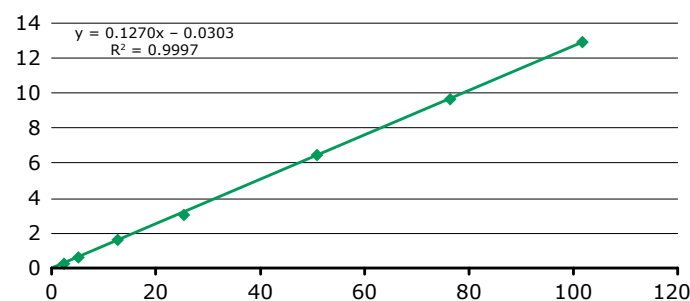
	Clobetasol propionate
STD 1	3.2246
STD 2	3.2257
STD 3	3.2512
STD 4	3.2835
STD 5	3.3183
Mean	3.2697
Standard Deviation	0.0401
(%) RSD	1.2

LOD & LOQ Clobetasol propionate

Conc. (µg/mL)	Mean Area (mAU*min)
1.0	0.1293
2.5	0.3217
5.1	0.6377
5.0	1.6213
25.4	3.0037
50.8	6.4720
76.2	9.6573
101.6	12.8877
STEYEX	0.0855
Slope	0.1
LOD (µg/mL)	2.22
LOQ (µg/mL)	6.73

Linearity (area/mAU*min) Clobetasol propionate

Conc. (µg/mL)	Mean Area (mAU*min)
1.0	0.1293
2.5	0.3217
5.1	0.6377
5.0	1.6213
25.4	3.0037
50.8	6.4720
76.2	9.6573
101.6	12.8877



Product list

	Cat. No.
Chromolith® HighResolution RP-18e, 100 x 2 mm I.D.	1.52322
Methanol gradient grade for liquid chromatography LiChrosolv® Reag. Ph Eur	1.06007
Water for chromatography (LC-MS grade) LiChrosolv®	1.15333
or tap fresh from an appropriate Milli-Q® system	172-0029
Millex® syringe filter units, disposable, Durapore® PVDF, pore size 0.45 µm, non-sterile	Z227447
Whitening cream	Local pharmacy
Cream without Clobetasol propionate	Local supermarket
Clobetasol propionate, Pharmaceutical Secondary Standard; Certified Reference Material	PHR1921

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

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