ORIGINAL ARTICLE

A New High-performance Liquid Chromatography Method Development for Cleaning Validation of Adapalene Active Pharma Ingredient

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Abstract

Aim: The analytical method has been developed to evaluate the efficacy of the cleaning procedure of all the equipment involved in the production of final active ingredients. The choice of the methodology is based on the production method and on the intrinsic properties of the products. For this validation, high-performance liquid chromatography (HPLC) method has been chosen. **Materials and Methods:** The HPLC chromatographic separations were achieved on (100 mm × 4.6 mm), 3.5 µm make: Phenomenex column employing acetonitrile and 0.5% orthophosphoric acid aqueous solution in the ratio of 35:65 as mobile phase with flow rate 1.0 mL/min was chosen. The column temperature was maintained at 30°C, and a detector wavelength of 230 nm was employed. **Results and Discussion:** The method was successfully validated by establishing system suitability, specificity, linearity, accuracy, limit of detection (LOD), and limit of quantification (LOQ) as per ICH guidelines. **Conclusion:** The method was completely validated showing satisfactory data for all methods - validated parameters tested. Satisfactory validation parameters such as linearity, recovery, precision, LOD, and LOQ were established by following ICH guidelines. Therefore, the proposed analytical procedure could be useful for regular monitoring, pharma manufacturing laboratories, and researchers.

Key words: Adapalene, cleaning validation, high-performance liquid chromatography, limit of detection, limit of quantification

INTRODUCTION

Adapalene is a synthetic naphthoic acid derivative with retinoid activity.[1] Adapalene is used in the treatment of acne vulgaris.[2] Acne vulgaris was not cured, but it will control the acne with adapalene. This results also will be clearly seen after the prolong usage.[3]

Oral antibiotics is used to kill the bacteria in the body, but on prolong usage, this antibiotics causes side effects and shows adverse affect on main organs in human body.[4] To overcome these situations, topical medicines are very useful in the field of dermatology.[1] At present, adapalene was available as gel in different compositions and combination with another molecule. Treatment with topical retinoids, such as adapalene, will lead to good results for acne vulgaris with less side effects and high efficacy. The accumulation of adapalene on skin is very low, decreased the risk of side effect, and works effectively on targeted areas.[5]

Cleaning validation is documented proof with high measure of assurance that one can always clean a system or piece of equipment to predetermined and suitable limits.[6] Cleaning validation is especially applicable to the cleaning of method manufacturing apparatus in pharmaceutical

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Received: 29-08-2016
Revised: 16-02-2017
Accepted: 23-02-2017

enterprise. It is integral to have effective cleaning programs in place because of regulatory requirements.[7] Cleaning is among the imperative strategies in pharmaceutical manufacturing. Equipment contamination may just come from any of the substances which have been in contact with the equipment surfaces.[8,9] It is crucial to restrict carryover of trace quantities of either active or different substances from one batch to yet another to preclude go-illness of the following product.[10,11] Consequently, equipment used in pharmaceutical manufacturing has got to be cleaned meticulously, and the cleaning approach used ought to be validated. In the pharmaceutical enterprise, just right manufacturing practices (good manufacturing practice [GMP]) require that the cleaning of drug manufacturing equipment be validated. Many unique validation methods can exhibit that the manufacturing gear is cleaned and just about free from residual energetic drug components and all cleaning agents.[12-14] Common analytical procedures in the validation procedure incorporate high-performance liquid chromatography (HPLC), spectrophotometry (ultraviolet/visible [UV/Vis]), and total organic carbon. HPLC and UV/Vis are categorized specific methods that identify and measure appropriate active and substances.

In the present study, a novel HPLC method was developed and successfully validated for adapalene. As on date, there were no research articles for cleaning validation of adapalene.

MATERIALS AND METHODS

Standards, reagents, and samples

The analytical standard of adapalene (99.3%) was obtained from Sigma-Aldrich. The HPLC grade solvents, i.e., orthophosphoric acid and acetonitrile were purchased from Rankem, New Delhi.

Experimental

HPLC chromatographic parameters

The HPLC-UV system used, consisted Shimadzu HPLC with LC-20AT pump and SPD-20A interfaced with LC solution software, equipped with a reversed-phase C18 analytical column of 100 mm × 4.6 mm, and particle size 3.5 µm (Phenomenex). Column oven temperature was maintained at 30°C. The injected sample volume was 10 µL. Mobile Phases A and B was acetonitrile and 0.5% orthophosphoric acid (35:65 [v/v]). The flow rate used was kept at 1.0 mL/min with a detector wavelength at 230 nm. The retention time of adapalene about 4.4 min.

Method validation

Method validation ensures analysis credibility. In this study, the parameters such as specificity and selectivity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) were considered. The accuracy of the method was determined is to verify the recovery and the release efficacy of the swabs and rinse used in the cleaning operation. Linearity was determined by different known concentrations (2.5, 5.0, 0.5, 10.0, 15.0, and 20.0 µg/mL) which were prepared by diluting the stock solution. The LOD (µg/mL) was determined as the lowest concentration giving a response of 3 times the baseline noise defined from the analysis of control sample. The LOQ (µg/mL) was determined as the lowest concentration of a given adapalene giving a response of 10 times the baseline noise.

RESULTS AND DISCUSSIONS

Specificity and selectivity

Procedure

The procedure was to demonstrate the discrimination of the analyte in the presence of others. Test samples containing each analyte then test sample without analyte (blank).

Take 10 mg of each product in each 100 ml volumetric flask and bring to volume with methanol. Take 10 ml of each solution in each 100 ml volumetric flask and bring to volume with methanol. Separately, inject once 10 µl of each solution.

Selectivity

Take 10 ml of each solution in a 100 ml volumetric flask and bring to volume to 100 ml with methanol (This solution contains 10 µg/mL of each substance). Inject 6 times 20 µl of this solution.

Since one product is utilized for this validation, six results of precision were used instead.

Linearity

The linearity was determined according to the ICH guidelines. The chosen concentration as 100% was 10 µg/ml of each product. The scheme carried out was the following.

Dilution scheme:

- Sample weight in 100 ml - Solution A
- 1 ml solution A in 100 ml - Solution B.

% MP	Concentration (µg/mL)	Stock solutions	Dilution (ml)
25	2.5	25 mg/100 ml	1-100 ml
50	5.0	50 mg/100 ml	1-100 ml
100	10.0	100 mg/100 ml	1-100 ml
150	15.0	150 mg/100 ml	1-100 ml
200	20.0	200 mg/100 ml	1-100 ml

Test solutions

25% solution

Take 25 mg of each product in a 100 ml volumetric flask and bring to volume with methanol. (Solution A). Take 1 ml in a 100 ml volumetric flask and bring to volume with methanol.

50% solution

Take 50 mg of each product in a 100 ml volumetric flask and bring to volume with methanol (Solution A1). Take 1 ml in a 100 ml volumetric flask and bring to volume with methanol.

100% solution

Take 100 mg of each product in a 100 ml volumetric flask and bring to volume with methanol (Solution A2). Take 1 ml in a 100 ml volumetric flask and bring to volume with methanol.

150% solution

Take 150 mg of each product in a 100 ml volumetric flask and bring to volume with methanol. (Solution A3). Take 1 ml in a 100 ml volumetric flask and bring to volume with methanol.

200% solution

Take 200 mg of each product in a 100 ml volumetric flask and bring to volume with methanol (Solution A4). Take 1 ml in a 100 ml volumetric flask and bring to volume with methanol.

The linearity solutions were injected thrice, and details were given in Table 1, and representative chromatogram was showed in Figure 1.

Precision: Repeatability

This was determined on six different solutions having a concentration of 10 µg/ml of each product (100%).

Dilution scheme:
- 100 mg in 100 ml - Solution A
- 1 ml Solution A in 100 ml - Solution B.

Precision solution

Take 100 mg of each product in a 100 ml volumetric flask and bring to volume with methanol. Take 1 ml in a 100 ml volumetric flask and bring to volume with methanol. The details were given in Table 2.

Precision: Intermediate

This was determined on six different solutions having a concentration of 10 µg/ml of each product, performed on different days, and using fresh mobile phase.

Dilution scheme:
- 100 mg in 100 ml - Solution A
- 1 ml Solution A in 100 ml - Solution B.

Table 1: Linearity details of adapalene

Set	Percent	Weight (mg)	Area injection 1	Normalized area	AV peak area	RSD %
1	25	25.0	187,891	187,891	188,263	0.73
2		25.2	188,609	187,112		
3		25.0	189,786	189,786		
1	50	50.0	386,934	386,934	391,438	1.33
2		49.8	395,561	397,150		
3		49.9	389,449	390,229		
1	100	100.0	807,672	807,672	811,808	0.46
2		99.9	814,234	815,049		
3		99.8	811,079	812,704		
1	150	150.0	1,219,277	1,219,277	1,222,055	0.20
2		149.7	1,221,401	1,223,849		
3		149.9	1,222,225	1,223,040		
1	200	199.8	1,647,350	1,648,999	1,660,813	0.43
2		199.8	1,659,969	1,661,631		
3		199.9	1,659,983	1,660,813		

AV: Average, RSD: Relation standard deviation

Slope	83,791
Intercept	–25667
R^2	0.9999

Precision solution

Take 100 mg of each product in a 100 ml volumetric flask and bring to volume with methanol. Take 1 ml in a 100 ml volumetric flask and bring to volume with methanol. The intermediate details were given in Tables 3 and 4.

Table 2: Repeatability details of adapalene

Injection	Weight Mg	Factor *N*	Area	Area *N*	Date
1	99.9	1.0010	809,405	810,215	19/12/06
2	100	1.0000	810,774	810,774	
3	99.9	1.0010	809,104	809,914	
4	100	1.0000	816,182	816,182	
5	100.1	0.9990	812,333	811,521	
6	100	1.0000	808244	808244	
Average	811142				
S	2700				
RSD %	0.33%				
Confidence	2160				

RSD: Relation standard deviation

Table 3: The intermediate details of adapalene on the 1st day

Injection	Weight Mg	Factor *N*	Area	Area *N*	Date
1	100	1.0000	815,092	815,092	20/07/2016
2	100.1	0.9990	810,325	809,515	
3	100	1.0000	810,701	810,701	
4	100.1	0.9990	810,016	809,207	
5	100	1.0000	811,278	811,278	
6	100	1.0000	816,003	816,003	

Accuracy

The purpose of determining accuracy is to verify the recovery and the release efficacy of the swabs and rinse used in the cleaning operation. The determination of the recovery factor is obtained using the following scheme:

- Transfer a known quantity of product, possibly dissolved in a volatile solvent, upon a surface which is similar to that used in the production plant. It is important to take care to distribute the product homogeneously on the surface
- Carefully eliminate the solvent from the surface to prevent loss of product from the surface
- Proceed to the mechanical cleaning of the surface (swab) or rinse as is described in the protocol using the identified solvent

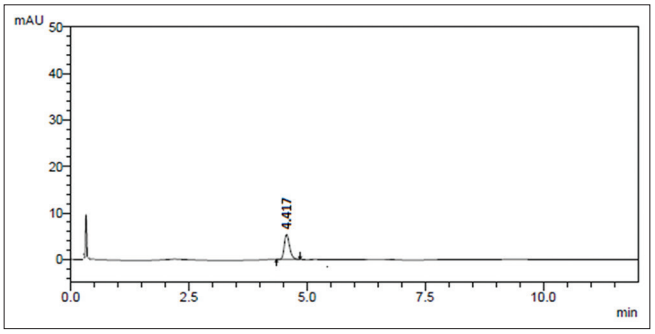

Figure 1: Representative chromatogram of linearity standard solution – 2.5 µg/mL

Table 4: The intermediate details of adapalene on the 2nd day

Injection	Weight Mg	Factor *N*	Area	Area *N*	Date
1	99.9	1.0010	809,405	810,215	19/07/2016
2	100	1.0000	810,774	810,774	
3	99.9	1.0010	809,104	809,914	
4	100	1.0000	816,182	816,182	
5	100.1	0.9990	812,333	811,521	
6	100	1.0000	808,244	808,244	
7	100	1.0000	815,092	815,092	20/07/2016
8	100.1	0.9990	810,325	809,515	
9	100	1.0000	810,701	810,701	
10	100.1	0.9990	810,016	809,207	
11	100	1.0000	811,278	811,278	
12	100	1.0000	816,003	816,003	
Average		811,554			
S		2701			
RSD %		0.33%			
Confidence		1528			

RSD: Relation standard deviation

- For standard solutions, one may use the means of peak areas obtained in precision - Intermediate precision results
- Extract with the swabs and determine the quantity of substance removed according to the analytical method. The percentage recovery obtained represents the recovery factor of the solvent to be used in the final calculation of the residual quantity of substance present in the equipment used for synthesis
- Repeat in triplicate the operation described with all surfaces with which product has come in contact.

Solution to be used: Use 1 ml of each solutions (Solution A1 50%; Solution A2 100%; and Solution A3 150%) prepared for the determination of linearity at 50%, 100%, and 150%. The swab and rinse details were given in Tables 5 and 6.

Accuracy: Glass lined

50% solution

- Swab - Take 1 ml of Solution A1. Extract the swab with 100 ml of methanol
- Rinse - Take 1 ml of Solution A1. Rinse with 100 ml of methanol.

100% solution

- Swab - Take 1 ml of Solution A2. Extract the swab with 100 ml of methanol
- Rinse - Take 1 ml of Solution A2. Rinse with 100 ml of methanol.

150% solution

- Swab - Take 1 ml of Solution A3. Extract the swab with 100 ml of methanol
- Rinse - Take 1 ml of Solution A3. Rinse with 100 ml of methanol.

The swab and rinse recovery details were given in Tables 7 and 8.

Accuracy: Steel

50% solution

- Swab - Take 1 ml of Solution A1. Extract the swab with 100 ml of methanol
- Rinse - Take 1 ml of Solution A1. Rinse with 100 ml of methanol.

100% solution

- Swab - Take 1 ml of Solution A2. Extract the swab with 100 ml of methanol
- Rinse - Take 1 ml of Solution A2. Rinse with 100 ml of methanol.

150% solution

- Swab - Take 1 ml of solution A3. Extract the swab with 100 ml of methanol
- Rinse -Take 1 ml of solution A3. Rinse with 100 ml of methanol.

The swab and rinse recovery details were given in Tables 9 and 10.

Accuracy: Rubber

50% solution

- Swab - Take 1 ml of Solution A1. Extract the swab with 100 ml of methanol
- Rinse - Take 1 ml of Solution A1. Rinse with 100 ml of methanol.

100% solution

- Swab - Take 1 ml of Solution A2. Extract the swab with 100 ml of methanol
- Rinse - Take 1 ml of Solution A2. Rinse with 100 ml of methanol.

Table 5: Swab table

%	Mg product	Volume	ml deposited	Volume extracted	Theoretic µg/ml
50	50	100	1	100	5
100	100	100	1	100	10
150	150	100	1	100	15

Table 6: Rinse table

%	Mg product	Volume	ml deposited	Volume extracted	Theoretic µg/ml
50	50	100	1	100	5
100	100	100	1	100	10
150	150	100	1	100	15

Table 7: Adapalene swab - glass lined

Name of the content	50%	100%	150%
Weight (mg)	50	100	150
Total dilution	100	100	100
µg/mL	5.00	10.00	15.00
µg deposited	500	1000	1500

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery %	AV recovery %
50% A	5.00	362,132	4.462	89.244	91.743
100% A	10.00	759,227	9.355	93.552	
150% A	15.00	1,125,195	13.865	92.431	
50% B	5.00	358,640	4.419	88.384	94.858
100% B	10.00	844,787	10.409	104.095	
150% B	15.00	1,121,112	13.814	92.096	
50% C	5.00	357,468	4.405	88.095	91.664
100% C	10.00	763,743	9.411	94.109	
150% C	15.00	1,129,540	13.918	92.788	
Mean recovery			92.75%		
RSD recovery			1.96%		

RSD: Relative standard deviation

Table 8: Adapalene rince - glass lined

Name of the content	50%	100%	150%
Weight (mg)	50	100	150
Total dilution	100	100	100
µg/mL	5.00	10.00	15.00
µg deposited	500	1000	1500

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery %	AV recovery %
50% A	5.00	386490	4.762	95.247	98.650
100% A	10.00	815246	10.045	100.455	
150% A	15.00	1220359	15.037	100.249	
50% B	5.00	385622	4.752	95.033	98.445
100% B	10.00	812096	10.007	100.067	
150% B	15.00	1220203	15.035	100.236	
50% C	5.00	385088	4.745	94.901	98.375
100% C	10.00	810679	9.989	99.892	
150% C	15.00	1221375	15.050	100.332	
Mean recovery			98.49%		
RSD recovery			0.15%		

RSD: Relative standard deviation

Table 9: Adapalene swab - steel

Name of the content	50%	100%	150%
Weight (mg)	50	100	150
Total dilution	100	100	100
µg/mL	5.00	10.00	15.00
µg deposited	500	1000	1500

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery %	AV recovery %
50% A	5.00	357,036	4.399	87.988	91.114
100% A	10.00	758,501	9.346	93.463	
150% A	15.00	1,118,607	13.784	91.890	
50% B	5.00	362,171	4.463	89.254	95.070
100% B	10.00	770,952	9.500	94.997	
150% B	15.00	1,228,991	15.144	100.958	
50% C	5.00	359,321	4.428	88.551	91.603
100% C	10.00	754,833	9.301	93.011	
150% C	15.00	1,135,135	13.987	93.248	
Mean recovery			92.60%		
RSD recovery			2.33%		

RSD: Relative standard deviation

Table 10: Adapalene rinse – steel

Name of the content	50%	100%	150%
Weight (mg)	50	100	150
Total dilution	100	100	100
µg/mL	5.00	10.00	15.00
µg deposited	500	1000	1500

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery %	AV recovery %
50% A	5.00	383,740	4.728	94.569	98.270
100% A	10.00	811,117	9.995	99.946	
150% A	15.00	1,220,926	15.044	100.295	
50% B	5.00	384,953	4.743	94.868	98.255
100% B	10.00	810,991	9.993	99.931	
150% B	15.00	1,216,924	14.995	99.967	
50% C	5.00	383,316	4.723	94.465	98.227
100% C	10.00	809,870	9.979	99.931	
150% C	15.00	1,222,472	15.063	99.967	
Mean recovery			98.25%		
RSD recovery			0.02%		

RSD: Relative standard deviation

150% solution

- Swab - Take 1 ml of Solution A3. Extract the swab with 100 ml of methanol
- Rinse - Take 1 ml of Solution A3. Rinse with 100 ml of methanol.

The swab and rinse recovery details were given in Tables 11 and 12.

LOQ and LOD

The LOQ is at least 1 µg/mL. Dilute 10 ml of linearity solution A at 100% in 100 ml of methanol. Inject 6 times 20 µl of this solution. The LOQ and LOD details were given in Tables 13 and 14, and representative LOQ chromatogram was showed in Figure 2.

The LOD is at least 0.25 µg/mL.

Inject 10 µl of solution used for the LOQ.

Calculations

The quantity of the active ingredient is determined according to the sampling procedure. The assay of the active ingredient is calculated by comparing the peak area, applying the formulas:

Table 11: Adapalene swab – rubber

Name of the content	50%	100%	150%
Weight (mg)	50	100	150
Total dilution	100	100	100
µg/mL	5.00	10.00	15.00
µg deposited	500	1000	1500

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery %	AV recovery %
50% A	5.00	362,316	4.464	89.289	91.662
100% A	10.00	752,275	9.270	92.696	
150% A	15.00	1,132,124	13.950	93.001	
50% B	5.00	358,487	4.417	88.346	92.048
100% B	10.00	766,667	9.447	94.469	
150% B	15.00	1,136,135	13.999	93.330	
50% C	5.00	367,896	4.533	90.665	92.252
100% C	10.00	758,176	9.342	93.423	
150% C	15.00	1,128,102	13.901	92.670	
Mean recovery			91.99%		
RSD recovery			0.33%		

RSD: Relative standard deviation

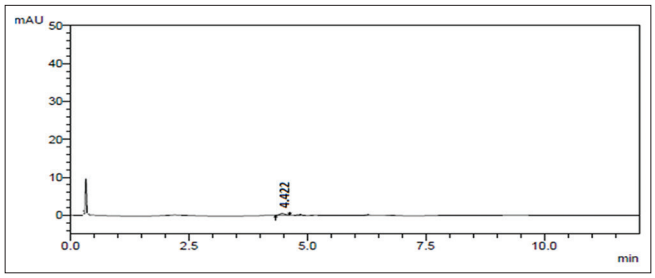

Figure 2: Limit of quantification level chromatogram of adapalene

Rinse

$$\frac{Ac*C}{As} = ug/mL \text{ in wash}$$

Where,
Ac: Area in sample solution,
As: Area in standard solution,
C: Concentration standard solution (µg/mL).

Calculation µg/mL in product based on rinse,

$$\frac{ug/mL \text{ product} * V}{1000 * kg \text{ (prod)}} = \text{ppm active ingredient}$$

Where,
V: Volume total solvent rinse (L),
kg: Quantity in kg of successive product,
1000: Conversion factor.

Swab

$$\frac{(Ac - Ab) * C \times Vestr}{As \times St} = ug/cm^2 \text{ in swab}$$

Where,
Ac: Area in sample solution,
Ab: Area blank extracted with swab,
As: Area in standard solution,
C: Concentration standard solution (µg/ml),
Vestr: Extraction solvent (ml),
St: Sampled surface (cm^2),

Calculation ppm in product based on swab:

$$\frac{ug/cm^2 \text{product} * S}{1000 * kg(prod) * R} = \text{ppm active ingredient}$$

Where,
S: Total surface of employed plant (cm^2),
kg: Quantity in kg of successive product,
1000: Conversion factor,
R: Recovery factor.

CONCLUSIONS

The method developed for quantitative determination of adapalene residues in clean samples. The method was completely validated showing satisfactory data for all

Table 12: Adapalene rinse – rubber

Name of the content	50%	100%	150%
Weight (mg)	50	100	150
Total dilution	100	100	100
µg/mL	5.00	10.00	15.00
µg deposited	500	1000	1500

Sample No.	Added (µg/ml)	Peak area	Found (µg/ml)	Recovery %	AV recovery %
50% A	5.00	383,892	4.730	94.607	98.355
100% A	10.00	810,547	9.988	99.876	
150% A	15.00	1,224,426	15.087	100.583	
50% B	5.00	384,508	4.738	94.758	98.427
100% B	10.00	811,009	9.993	99.933	
150% B	15.00	1,224,508	15.088	100.590	
50% C	5.00	383,341	4.724	94.471	98.211
100% C	10.00	809,717	9.977	99.774	
150% C	15.00	1,222,070	15.058	100.389	
Mean recovery			98.33%		
RSD recovery			0.11%		

RSD: Relative standard deviation

Table 13: LOQ details of adapalene

Set	Area	Found (µg/mL)	Recovery %
1	82053	1.011	101.106
2	82501	1.017	101.658
3	82159	1.012	101.237
4	82011	1.011	101.054
5	81526	1.005	100.457
6	82097	1.012	101.160
Average	82057.83	1.011	101.112
SD	314.08	0.004	0.387
RSD	0.38	0.383	0.383

LOQ: Limit of quantification, SD: Standard deviation, RSD: Relative standard deviation

Table 14: LOD details of adapalene

Sample	Area	Found (µg/mL)	Recovery %
Adapalene	19,706	0.24	97.127%

LOD: Limit of detection

methods - validated parameters tested. The mobile phase composition of acetonitrile and 1% H_3PO_4 in water showed good separation and resolution. Satisfactory validation parameters such as linearity, recovery, precision, LOD, and LOQ were established by following ICH guidelines.[15] Therefore, the proposed analytical procedure could be useful for regular monitoring, pharma manufacturing laboratories, and researchers.

ACKNOWLEDGMENT

The authors are thankful to the Dr. B. Gowtham Prasad, SVV University, for providing necessary facility to conduct the laboratory experiment.

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Source of Support: Nil. **Conflict of Interest:** None declared.