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RP-HPLC Method Development and Validation for Simultaneous Estimation of Nadifloxacin and Adapalene in Bulk and Dosage Form

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ABSTRACT:

A simple, accurate, rapid and precise isocratic high-performance liquid chromatographic (HPLC) method has been developed and validated for simultaneous estimation of Nadifloxacin and Adapalene in pure powder and formulation. The HPLC separation was achieved on a Sheisedo-C18 (250mm x 4.6mm, 5 µm) using Acetonitrile: Methanol: Water (pH-2.8) (35:45:20 v/v) as mobile phase at flow rate of 1.0 ml/min. The calibration plot showed good linear relationship with r2=0.999 for NAD and r2=0.999 for ADA in concentration range of 100-500 µg/ml and 10-50 µg/ml respectively. Limit of detection (LOD) and Limit of quantification (LOQ) were found to be 4.0984µg/ml and 12.4196µg/ml for Nadifloxacin and 1.1237µg/ml and 3.4053µg/ml for Adapalene. Assay of Nadifloxacin found to 99.37±0.4064% and Adapalene found to 99.33±0.4041%. The method was validated as per ICH guideline. The method was successfully applied for routine analysis of Nadifloxacin and Adapalene in pure powder and gel formulation.

KEYWORDS: Adapalene; Nadifloxacin; RP-HPLC method; Validation.

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1. INTRODUCTION:[1 -7]

Nadifloxacin is a potent, broad-spectrum, quinolone agent approved for topical use in acne vulgaris and skin infections. The chemical name for Nadifloxacin (±)-9-Fluoro-6,7-dihydro-8-(4-hydroxy piperidino)-5-methyl 1 oxo 1H,5H benzo(ii)quinolizine-2-carboxylic acid. Nadifloxacin inhibits the enzyme DNA gyrase that is invovled in bacterial DNA synthesis and replication, thus inhibiting the bacterial multiplication. Nadifloxacin in addition to determine a therapeutic antibacterial action can have a sebostatic and anti inflammatory action, thus contributing to the clinical condition of the patient.

Adapalene is a synthetic topical retinoid and a naptholic acid derivative.[1, 2]. The chemical name for Adapalene is 6-[-3-(1-adamantyl)-4-methoxy-phenyl] 2-naphthoic acid. Adapalene inhibit keratinocyte differentiation. This inhibition of keratinocyte differentiation and proliferation is responsible for adapalene'scomedolytic effect. It has both exfoliating and anti-inflammatory effects. In an in vivo study, adapalene's ability to reduce comedo formation was demonstrated by a 50-60% reduction in comedo counts compared with vehicle.

Literature surveys of Nadifloxacin and Adapalene revealed that there are analytical methods such as spectrophotometric, HPLC, and HPTLC, which are reported either alone or in combination with other components. Combination therapy of retinoid (Adapalene) and antibacterial agent (Nadifloxacin) is effective against acnes and it is available on the market as a topical gel formulation. Individual NAD and ADA gel formulations are also available on the market.

The aim of the present study was to develop and validate a RP-HPLC method for the simultaneous estimation of NAD and ADA in bulk or in topical gel formulations. The method was thoroughly validated for method precision, accuracy, linearity, LOD and LOQ, specificity, and robustness as per ICH guidelines.

2. MATERIALS AND METHODS

2.1. Equipment:

Chromatographic separation was performed on HPLC system consist of model Shimadzu having UV-Vin detector and injector with 10µl loop volume. LC solution software was applied for data collecting and processing.

2.2. Reagents and chemicals:

Acetonitrile and methanol of HPLC grade were procured from Ran Kem lab ltd. The pure Nadifloxacin was obtained from Amideep Pharmaceutical (Gift Sample) and pure Adapalene was obtained from Aarti Industries (Gift Sample).

2.3. HPLC Conditions:

A SheisedoC_{18} (250*4.6 mm, 5µm) column was used as the stationary phase. A mixture of Acetonitrile, methanol and water in the ratio of (35 : 45 : 20 %v/v) was used as a mobile phase and pH 2.8 adjusted with Ortho phosphoric acid. It was filtered through 0.45 µ membrane filter and degassed. The mobile phase was pumped at 1.0 ml/min. The eluents were monitored at 271nm. The injection volumes of sample and standard were 10 µl.

2.4. Preparation of stock and standard solutions:

100 mg of Nadifloxacin was weighed and transferred to a 100 ml volumetric flask and dissolved in Methanol and sonicated for about 10 min. Volume was made up to the mark with Methanol to give a solution containing 1000 µg/ml Nadifloxacin. 10 mg of Adapalene was accurately weighed and transferred to a 100 ml volumetric flask and dissolved in Methanol and sonicated for about 10 min. Volume was made up to the mark with Methanol to give a solution containing 100 µg/ml Adapalene. The solution was filtered through 0.45 micron meter nylon membrane filter paper. Working standard solution was prepared by making various dilutions of the drug solution from the stock solution. Six sets of the drug solution were prepared in the mobile phase containing a concentration of 100-600 µg/ml for nadifloxacin and concentration of 10-60 µg/ml for Adapalene. Each of these drug solution (10µl) was injected into the column and the peak area and retention time were recorded.

2.5. ASSAY OF TABLET FORMULATION:

One gm of gel equivalent to 10 mg Nadifloxacin and 1 mg Adapalene transferred to 100 ml of volumetric flask and add 30 ml Methanol and sonicated for 10 min. The flask was shaken and volume was made up to the mark with mobile phase.Mixed solution containing 100 µg/ml of Nadifloxacin and 10 µg/ml of Adapalene. Above solution was filtered through whatman filter paper. This Test solution was injected and chromatogram was recorded for the same. Concentrations of Nadifloxacin and Adapalene in the formulation were calculated by comparing area of the sample with that of standard. The percentage assay of individual drug was calculated.

2.6. METHOD VALIDATION[14 – 15]

The developed method was validated as per ICH guidelines for its System suitability, linearity, accuracy, precision, robustness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in Table.

2.6.1. System Suitability

System suitability tests are an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. System Suitability was performed on standard solution and system suitability parameters were calculated at the start of study for each parameter. The test includes Parameters like Number of Theoretical Plates, Resolution, Retention time and tailing factor and recorded

2.6.2. Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of Nadifloxacin and Adapalene at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance vs concentration of the drug. The responses were found to be linear in the range 100-600 µg/ml and 10-60 µg/ml for Nadifloxacin and Adapalene. The data was given in Table 4.

2.6.3. Accuracy

Recovery studies were carried out by addition of standard drug to the sample at 4 different concentration levels (0%, 80%, 100% and 120%) taking into consideration percentage purity of added bulk drug samples. These solutions were subjected to re-analysis by the proposed method and Results are calculated.

2.6.4. Precision

A) Repeatability

Mixed solutions containing 200, 300, 400 µg/ml Nadifloxacin and 20, 30, 40 µg/ml Adapalene were prepared and Chromatogram were recorded. Area was measured of the same concentration solution three times and %RSD was calculated.

B) Intraday precision

Mixed solutions containing 200, 300, 400 µg/ml Nadifloxacin and 20, 30, 40 µg/ml Adapalene were analyzed three times on the same day and % R.S.D was calculated.

C) Interday precision

Mixed solutions containing 200, 300, 400 µg/ml Nadifloxacin and 20, 30, 40 µg/ml Adapalene were analyzed on three different days and % R.S.D was calculated.

2.6.5. Limit of detection and Limit of Quantification

From the linearity curve equation, the standard deviation (SD) of the intercepts (response) was calculated. The limit of detection (LOD) and limit of quantitation (LOQ) of the drug was calculated by using the following equation designated by International Conference on Harmonization (ICH) guideline:

$LOD = 3.3\,\boldsymbol{\sigma} / S$ and $LOQ = 10\,\boldsymbol{\sigma} / S$

Where, σ = the standard deviation of the response

S = slope of the calibration curve

2.6.6. Robustness

The robustness of the method was established by making deliberate minor variations in the parameters like pH of mobile phase:±0.2, Flow rate : ±0.2 ml/min, Change in the ratio of component in the mobile phase: ± 2%. The effects of changes observed were recorded.

3. RESULT AND DISCUSSION

3.1. System suitability

System suitability and chromatographic parameters were validated such as resolution, theoretical plates, and tailing factor was calculated. The result is given in Table 3.

3.2. Linearity

The calibration curve showed (Fig.3, 4, 5) good linearity in the range of 100-600µg/ml, for Nadifloxacin with correlation coefficient (r^2) of 0.999 and 10-60µg/ml for Adapalene with correlation coefficient (r^2) of 0.999. A typical calibration curve has the regression equation of y =29.88x + 37.11 for Nadifloxacin and y =100.4x + 34.19 for Adapalene.

3.3. Recovery

At each concentration, sample was injected thrice to check repeatability and from the RSD values it was analyzed that the method was accurate as % recovery values found to be in the range of 99.65-100.07 % at three different concentrations 200, 300 and 400µg/ml for Nadifloxacin and 99.65-100.16 % at three different concentrations 20, 30 and 40µg/ml for Nadifloxacin. The results are given in Table 6, 7.

3.4. Precision

Intraday precision was carried out using test samples prepared and analyzed on the same day. Interday precision was assessed by analysis of the same solutions on consecutive days. The low % RSD values below 2 indicate that the method is precise. Repeatability also performed. The results are given in table 8, 9, 10, 11.

3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were found to be 12.41 µg/ml and 4.098 µg/ml for Nadifloxacin and 3.40 µg/ml and 1.123 µg/ml for Adapalene estimated by using the standard formulas. The low values of LOD and LOQ illustrate that the developed method was sensitive, accurate and precise as it can detected and quantify with very low concentration. The result is given in Table 12.

3.6. Robustness

Small deliberate changes in chromatographic conditions such as change in mobile phase ratio (± 2 %), change in pH (±2 units) and flow rate (± 2 units) were studied to determine the robustness of the method. The results were in factor of (% RSD< 2%) the developed RP-HPLC method for the analysis of Nadifloxacin and Adapalene. The results are given in Table 13, 14**.**

3.7. DISCUSSION

RP-HPLC method was found to be linear over the range of 100-600 µg/ml for Nadifloxacin and 10-60 µg/ml for Adapalene. The method has been validated for linearity, accuracy and precision, LOD, LOQ and system suitability according to ICH guideline.

4. CONCLUSION

This study is a typical example of the development of an assay method following ICH guidelines. A new isocratic RP-HPLC method has been developed and validated for determination of Nadifloxacin and Adapalene in the gel dosage form. The results of the validation studies showed that the RP-HPLC method possesses significant linearity, precision, accuracy, specificity, sensitivity, high efficiency and resolution, and no interference from the excipients, as were demonstrated. The proposed method was successfully applied and is suggested for the quantitative analysis of Nadifloxacin and Adapalene in combined pharmaceutical formulations for QC, where economy and time are essential and to assure therapeutic efficacy.

5. REFERENCES

1. Tripathi K., Essential of Medical Pharmacology; 6th Edition; Jaypee Brother Medical Publication; 2010, 688-689.
2. Rang H. et al, Pharmacology; 6th Edition; Churchill Livingstone; Elsevier Science Ltd; 2007, 661.
3. James S., Encyclopedia of Pharmaceutical Technology; 3rd Edition; 2007, Vol-5,3460-3475.
4. Sharma B., Instrumental Method of chemical analysis; 21th Edition; Goel Publishing Housing, Krishna Prakashan Ltd, 2002, 3-10.
5. Skoog D. et al, Fundamentals of Analytical Chemistry; 8th Edition; Thomson Brook/cole, 2005, 301,739-741.
6. Drug information: Nadifloxacin http://en,wikipedia.org/wiki/Nadifloxacin
7. Drug information: Adapalene http://en.wikipedia.org/wiki/Adapalene
8. Devhadrao N. et al, "Analytical method development and validation of s-nadifloxacin in pure form by HPLC."*International Journal of Pharmaceutical and clinical research*, 2014, 6(1), 63-67.
9. Ayyagari R. et al, "Analytical method development and validation of nadifloxacin by high performance liquid chromatography."*International Journal of Pharmacy and Pharmaceutical sciences*, 2014, 6(9), 344-346.
10. Laura A. et al, "A simple HPLC DAD method for determination of adapalene in topical gel formulation."*Journal of chromatographic science*, 2011, 49, 796-800.
11. Mailvelan R. et al, "HPLC method development and validation for the estimation of adapalene in Pharm. formulations."*Asian Journal of pharmaceutical analysis and medicinal chemistry*, 2013, 1(3), 166-171.
12. Rohit H. et al, "A new RP-HPLC method for estimation of clindamycin and adapalene in gel formulation: Development and validation consideration."*The Thai Journal of Pharmaceutical Sciences*,2014, 38(1), 1-56.
13. Nash R. and Watcher A., Pharmaceutical Process Validation, Marcel Dekker Inc.; New York; 2003; 507-522.
14. ICH, Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Geneva, 2005.
15.

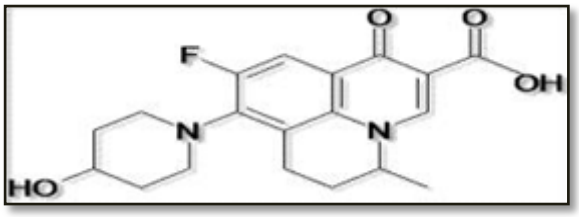


Figure 1- Structure of nadifloxacin

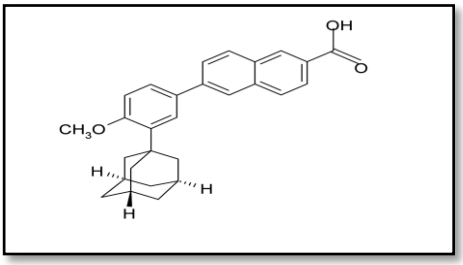


Figure 2- Structure of Adapalene

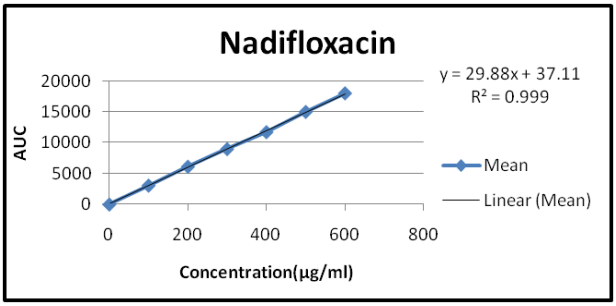


Figure -3 Calibration curve of Nadifloxacin

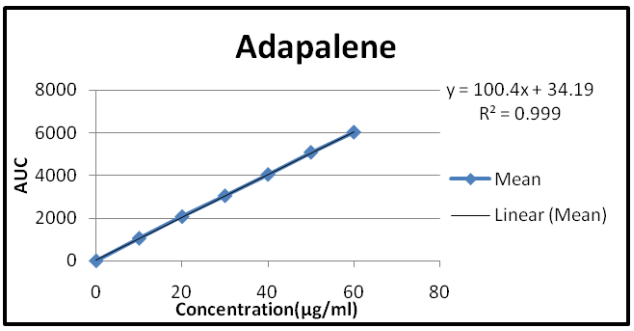


Figure-4 Calibration curve of Adapalene

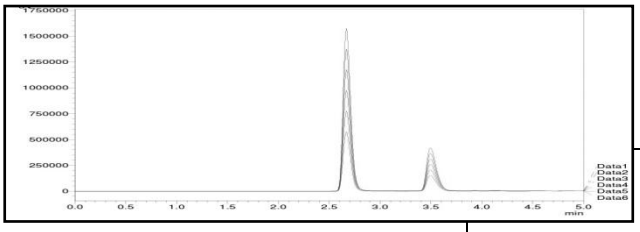


Figure 5- Calibration curve for Nadifloxacin and Adapalene

Table: 1 Calibration Data of Nadifloxacin and Adapalene

Nadifloxacin		Adapalene	
Conc. (µg/ml)	Area	Conc. (µg/ml)	Area
0	0	0	0
100	3068.948 ± 5.4027	10	1054.411 ± 9.7812
200	6129.447 ± 6.5710	20	2069.906 ± 7.5185
300	8998.46 ± 8.8347	30	3047.821 ± 8.0034
400	11745.742 ± 8.1241	40	4044.68 ± 6.3946
500	15030.082 ± 7.1711	50	5084.200 ± 9.8865
600	18047.14 ± 8.9258	60	6028.72 ± 9.1699

Figure -3 Calibration curve of Nadifloxacin

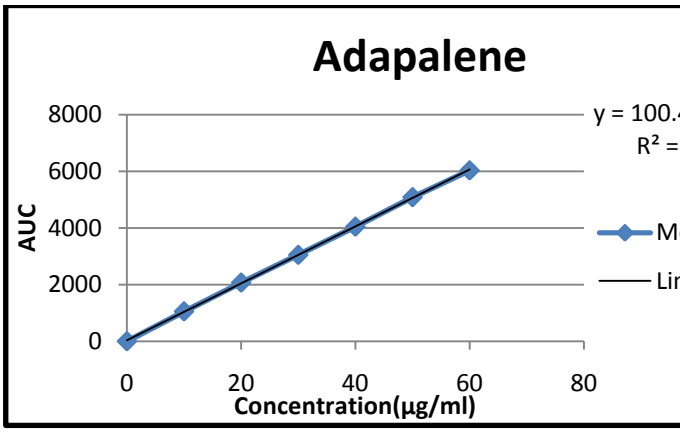


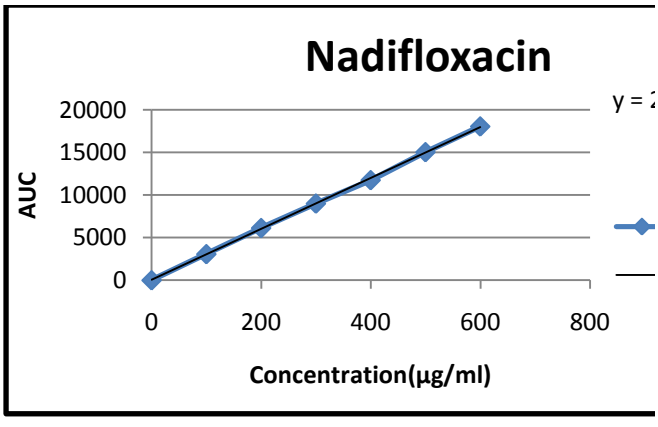
Figure-4 Calibration curve of Adapalene

Table: 2 Assay Data of Nadifloxacin

	Actual conc. of Drug (µg/ml)	Conc. of Drug Found (µg/ml)	% of Drug found	Avg. of % Drug found	SD
Nadifloxacin	100	98.91	98.91	99.37	0.4064
	100	99.54	99.54		
	100	99.67	99.67		
Adapalene	10	9.94	99.4	99.33	0.4041
	10	9.89	98.9		
	10	9.97	99.7		

Table: 3 System Suitability tests

Sr. No.	System suitability parameter	Nadifloxacin	Adapalene	Spec per
1	Retention time (min)	2.664	3.490	-
2	Resolution (R)	4.883		>1.5
3	Theoretical plate number (N)	5045.909	5471.532	N
4	Tailing factor (T)	1.361	1.539	Not



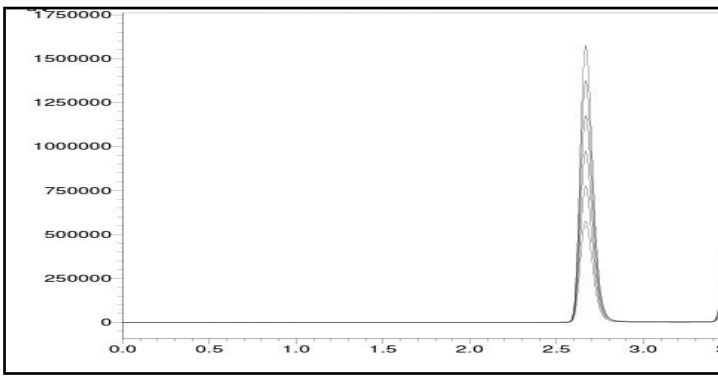


Figure 5- Calibration curve for Nadifloxacin and Adapalene

Table: 4 Linearity data for Nadifloxacin

Conc. (µg/ml)	Area. Mean ± S.D
0	0
100	3067.732667 ± 7.0795
200	6127.246667 ± 6.7850
300	8999.647667 ± 9.7929
400	11743.977 ± 9.1198
500	15031.77833 ± 9.3019
600	18050.45233 ± 6.1225

Table: 5 Linearity data for Adapalene

Conc. (µg/ml)	Area. Mean ± S.D
0	0
10	1052.687 ± 6.799
20	2067.739 ± 7.7117
30	3045.067 ± 9.7388
40	4043.577 ± 8.3393
50	5078.972 ± 8.4242
60	6031.441 ± 8.6131

Table: 6 Accuracy Data of Nadifloxacin

Target Conc.	Spiked Conc.	Final Conc.	Conc. Obtained	% Assay	Mean	SD	%RSD
20	16	36	35.67	99.08	99.67	1.7438	1.7495
20	16	36	35.39	98.30			
20	16	36	36.59	101.63			
20	20	40	40.06	100.15	100.10	0.1421	0.1420
20	20	40	40.09	100.22			
20	20	40	39.98	99.95			
20	24	44	44.78	101.77	99.69	1.8396	1.8352
20	24	44	43.26	98.32			
20	24	44	43.56	99.00			

Table: 7 Accuracy Data of Adapalene

Target Conc.	Spiked Conc.	Final Conc.	Conc. Obtained	% Assay	Mean	SD	%RSD
2	1.6	3.6	3.59	99.72	99.44	1.2729	1.2800
2	1.6	3.6	3.62	100.56			
2	1.6	3.6	3.53	98.06			
2	2.0	4.0	4.01	100.25	100.17	1.1273	1.1254
2	2.0	4.0	3.96	99.00			
2	2.0	4.0	4.05	101.25			
2	2.4	4.4	4.43	100.68	99.54	1.2654	1.2711
2	2.4	4.4	4.32	98.18			
2	2.4	4.4	4.39	99.77			

Table: 8 Repeatability Data for Nadifloxacin

Concentration(µg/ml)	Area Mean ± S.D. (n=3)	%RSD
200	6130.047 ± 2.3783	0.03879
300	9003.856 ± 6.7620	0.07510
400	11740.59 ± 5.6127	0.04780

Table: 9 Repeatability Data for Adapalene

Concentration(µg/ml)	Area Mean ± S.D. (n=3)	%RSD
20	2070.318 ± 3.8835	0.1875
30	3049.024 ± 7.5257	0.2468
40	4047.604 ± 4.7023	0.1161

Table: 10 Intraday and Interday precision Data for Nadifloxacin

Concentration (µg/ml)	Intraday Peak Area. ± S.D. (n=3)	%R.S.D.	Interday Peak Area ± S.D. (n=3)	%R.S.D.
200	6132.724 ± 4.7071	0.0767	6127.247 ± 6.7850	0.1107
300	8994.941 ± 7.9984	0.0889	8999.648 ± 9.7929	0.1088
400	11740.59 ± 5.6127	0.0478	11743.98 ± 9.1198	0.0776

Table: 11Interday precision Data for Adapalene

Concentration (µg/ml)	Intraday Peak Area. ± S.D. (n=3)	%R.S.D.	Interday Peak Area ± S.D. (n=3)	%R.S.D.
20	2073.656 ± 6.0604	0.2922	2067.74 ± 7.7117	0.3729
30	3049.688 ± 5.3453	0.1752	3045.067 ± 9.7388	0.3198
40	4037.051 ± 3.8031	0.0942	4043.578 ± 8.3393	0.2062

Table: 12 LOD and LOQ Data for Nadifloxacin and Adapalene

Drugs	LOD (µg/ml)	LOQ (µg/ml)
Nadifloxacin	4.0984	12.419
Adapalene	1.1237	3.4053

Table: 13 Robustness Data for Nadifloxacin

Sr. No.	Concentration(300µg/ml)					
	pH		Flow Rate		Mobile Phase	
	+0.2 units	-0.2units	+0.2units	-0.2units	+0.2units	-0.2units
1	9010.498	9008.98	9004.089	9011.486	8995.125	8994.726
2	8996.043	9018.484	8994.576	9008.762	9010.756	9014.185
3	9006.179	9013.146	9012.864	8995.862	9008.725	9007.458
Mean	9004.24	9013.537	9003.843	9005.37	9004.868	9005.456
S.D.	7.4200	4.7640	9.1464	8.3460	8.4991	9.8827
%RSD	0.08240	0.05285	0.1015	0.0926	0.0943	0.1097

Table: 14 Robustness Data for Adapalene

Sr. No.	Concentration(300µg/ml)					
	pH		Flow Rate		Mobile Phase	
	+0.2 units	-0.2units	+0.2units	-0.2units	+ 2%	-2%
1	3056.153	3041.156	3049.762	3037.843	3036.829	3044.672
2	3037.891	3056.183	3051.866	3043.953	3052.183	3037.853
3	3043.424	3049.045	3062.054	3039.046	3044.348	3056.286
Mean	3045.822	3048.794	3054.560	3040.280	3044.453	3046.270
S.D.	9.3643	7.5166	6.5741	3.2367	7.6775	9.3198
%RSD	0.3074	0.2465	0.2152	0.1064	0.2521	0.3059

Table: 15 Summary of Validation Parameters for NAD and AD

PARAMETER	Nadifloxacin	Adapalene
Range(µg/ml)	100-600	10-60
Equation (y = mx + c)	29.88x + 37.11	100.4x + 34.19
Correlation coefficient	0.999	0.999
LOD (µg/ml)	4.0984	1.1237
LOQ (µg/ml)	12.4196	3.4053
Repeatability (%RSD)	0.03879-0.0751	0.1161-0.2468
Interday precision (%RSD)	0.0776-0.1107	0.2062-0.3729
Intraday precision (%RSD)	0.0478-0.0889	0.0942-0.2922
Robustness	0.0528-0.1097	0.1064-0.3074
% Recovery	99.65-100.07	99.65-100.16
Assay	99.37%	99.33%