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STABILITY INDICATING SIMULTANEOUS VALIDATION OF AZELAIC ACID, MINOXIDIL AND TRETINOIN WITH FORCED DEGRADATION BEHAVIOR STUDY BY RP-HPLC IN PHARMACEUTICAL DOSAGE FORM.

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ABSTRACT
A simple, precise, and accurate RP-HPLC method has been developed and validated for the simultaneous assay of Azelaic acid, Minoxidil and Tretinoin in topical gel. Isocratic RP-HPLC method was developed on BDS hypersil C18, (250mm×4.6mm internal diameter, 5µ particle size) using mobile phase as 0.05M Potassium Dihydrogen Phosphate (pH-4.0): Methanol (80:20v/v) at a flow rate of 1.0 mL/min and the detection was carried out at 243nm using tunable absorbance detector (Waters 486). Forced degradation study was carried out by acid degradation, base degradation, thermal degradation, oxidation of the drug. The method was validated for linearity, precision, accuracy and robustness. The method was found to be linear in the concentration range of 7.5-37.5 μg/mL with correlation coefficient of 0.9995 for Azelaic acid, 25-125 μg/mL with correlation coefficient of 0.9999 for Minoxidil and 0.5-2.5 μg/mL with correlation coefficient of 0.9996 for Tretinoin. Degradation products produced as a result of stress studies did not interfere with the detection of Azelaic acid, Minoxidil and Tretinoin; therefore, the assay can be considered to be stability indicating.

KEYWORDS: HPLC, Azelaic acid, Minoxidil, Tretinoin, Validation, Forced degradation.

INTRODUCTION

Azelaic Acid's empirical formula is $C_9H_{16}O_4$ and its IUPAC name is nonanedioic acid. Figure 1 shows chemical structure of Azelaic acid. Azelaic acid is an organic compound. This saturated dicarboxylic acid exists as a white powder. It is found in wheat, rye, and barley. It is a precursor to diverse industrial products including polymers, plasticizers, as well as being a component of a number of hair and skin conditioners. Minoxidil's empirical formula is $C_9H_{15}N_5O$ and its IUPAC name is 6-Piperidin-1-ylpyrimidine-2,4-diamine 3-oxide. Figure 2 shows chemical structure of Minoxidil. Minoxidil is an antihypertensive vasodilator medication. It also slows hair loss and promotes hair regrowth in some people. Now off-patent, it is available over the counter for the treatment of androgenic alopecia. Tretinoin's empirical formula is $C_{20}H_{28}O_2$ and its IUPAC name is (2E,4E,6E,8E)-3,7-Dimethyl-9-(2,6,6-trimethylcyclohexen-1-yl)nona-2,4,6,8-tetraenoic acid. Figure 3 shows the chemical structure of Tretinoin. Tretinoin is the pharmaceutical form of retinoic acid. One of several retinoids, it is the carboxylic acid form of vitamin A and is also known as all-trans retinoic acid (ATRA). It is a first generation topical retinoid commonly used topically to treat acne vulgaris. It is also used orally to treat acute promyelocytic leukemia (APL). Its isomer, isotretinoin, is also an acne drug.

Literature survey reveals that quantitative analysis of Azelaic acid, Minoxidil and Tretinoin have been done separately or in combination of two and in combination of other drugs but no method is reported for the simultaneous estimation of Azelaic acid, Minoxidil and Tretinoin in combined dosage form. The present study involved the development and validation of RP-HPLC method for the estimation of Azelaic acid, Minoxidil and Tretinoin in combined pharmaceutical dosage form (topical gel) and their forced degradation study [1-4].

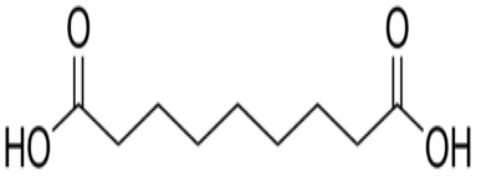

FIGURE 1: Structure of Azelaic acid

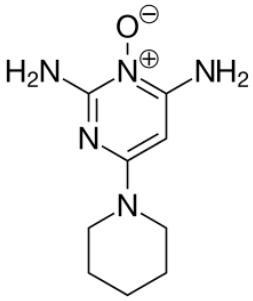

FIGURE 2: Structure of Minoxidil

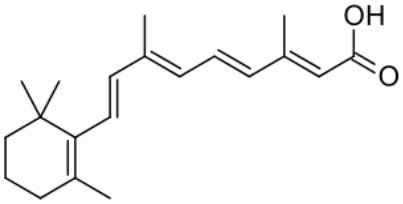

FIGURE 3: Structure of Tretinoin

MATERIAL AND METHODS

Instruments

The liquid chromatographic system consists of Waters series M510 equipped with a tunable absorbance detector (Waters 486), HPLC pump (Waters 510), and manual injector rheodyne valve with 20 μL fixed loop. The analytes were monitored at 243 nm. Chromatographic analysis was performed on Thermo scientific BDS hypersil C18, (250mm × 4.6mm internal diameter, 5µ particle size). All the drugs and chemicals were weighed on Citizen electronic balance. Chemiline India pH meter and Toshcon Ultrasonicator was used.

Chemicals and reagents

Methanol was of HPLC grade obtained from Merck Ltd., Mumbai. Water was of HPLC grade prepared by triple distillation method. Potassium Dihydrogen Phosphate, Ortho Phosphoric Acid (OPA), Sodium Hydroxide (NaOH), Hydrogen Peroxide (H_2O_2) and Hydrochloric Acid (HCl) were of AR grade and were obtained from Merck, Mumbai India. Azelaic acid, Minoxidil and Tretinoin reference standards obtained as gift samples from Zydus Cadila, Ahmedabad. Minokem N 5% Topical gel containing 15mg of Azelaic acid, 50mg of Minoxidil and 0.1mg of Tretinoin manufactured by Alkem Laboratories Ltd. was procured from local market.

HPLC Conditions

The mobile phase consisted of 0.05M Potassium Dihydrogen Phosphate (pH-4.0): Methanol (80:20v/v).The mobile phase was prepared freshly and it was sonicated by using Toshcon Ultrasonicator for 5 min before use. BDS hypersil C18, (250mm×4.6mm internal diameter, 5µ particle size) was used and it was was equilibrated for at least 30 min with the mobile phase flowing through the system. The column and the HPLC system were kept at ambient temperature. The eluent was monitored by UV detection at 243 nm. Analysis was done at flowrate of 1.0ml/min with 20µl volume of injection. All data were analyzed by using Empower 3 software.

Preparation of Mobile Phase

The mobile phase was prepared by mixing 0.05M Potassium Dihydrogen Phosphate (pH-4.0) and Methanol in the ratio of (80:20%v/v). The solution was then filtered through 0.45 microns membrane filter and degassed.

Preparation of 0.05M Potassium Dihydrogen Phosphate (pH-4.0)

Take about 6.8gm Potasium dihydrogen phosphate into a 1000ml beaker. Add 800ml water and dissolve. Adjust ph 4.0 of this solution with 1% Orthophosphoric acid. Make up volume upto 1000ml with water.

Preparation of standard stock solution

Standard stock solution of Azelaic Acid, Minoxidil and Tretinoin were prepared by accurately weighing 15mg, 50mg and 10mg respectively and dissolving them separately in 100ml with methanol to prepare solution of 150μg/mL, 500μg/mL and 100μg/mL. The solutions of Tretinoin was further diluted by taking 10 ml of standard stock solution and diluted upto 100 ml with methanol separately to prepare solution of 10μg/mL.

Preparation of working standard solution

Add 1ml each of standard stock solution of Azelaic acid, Minoxidil and Tretinoin in 10 ml volumetric flask and volume make up to 10ml with methanol.

Preparation of sample stock solution

Weight about 1gm topical gel (equivalent to 15mg of Azelaic acid, 50mg of Minoxidil and 0.1mg of Tretinion) into a 100ml volumetric flask. Add 60ml methanol and put this volumetric on water bath at 60°C for 15 minutes then allow cooling at room temperature. Shake for 15 minutes. Make up volume with methanol upto 100ml. Filter this solution.

Preparation of sample working solution

Take 1ml from sample stock solution into a 10ml volumetric flask, add 1ml Tretinoin standard stock solution and make up with mobile phase to prepare a solution of Azelaic acid 15mcg/ml, Minoxidil 50mcg/ml and Tretinoin 1.1mcg/ml.

Forced Degradation Study

Preparation of solution for acid degradation

Acid decomposition study was performed by keeping the working solution of all three drugs (1 ml) in 2 ml of 0.1N HCl for 4 hrs. After 4 hrs solution neutralized with 2ml 0.1N NaOH and finally made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

Preparation of solution for basic degradation
Alkali decomposition study was performed by keeping the working solution of all three drugs (1 ml) in 2 ml of 0.1N NaOH for 2.5 hrs. After 2.5 hrs solution neutralized with 2 ml of 0.1N HCL and finally made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected in to HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

Preparation of solution for oxidative degradation
Oxidative decomposition study was performed by keeping the working solution of all three drugs (1 ml) in 2 ml 3% H_2O_2 for 4 hrs. After 4 hrs volume made up to 10 ml with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

Preparation of solution for thermal degradation
Thermal decomposition study was performed by refluxing the working solution of all three drugs (1 ml) for 4 hrs at 105 ºC. After 4 hrs volume made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

Preparation of solution for UV degradation
UV degradation was performed by exposing the working solution of all three drugs (1ml) to Sunlight for 3.5 hours. After 3.5 hours volume made up to 10 ml volume with mobile phase, sonicated and filtered through 0.45µm membrane filter paper and injected into HPLC system. Degradation samples were prepared as blank sample, separate standard samples and combined sample of all three drugs were prepared.

Determination of λ max
The UV spectra of standard stock solutions of Azelaic acid, Minoxidil and Tretinoin was taken between the wave length range of 200-400nm using methanol as blank. The λ max was found to be 226.90nm, 240.34nm and 243.80nm for Azelaic acid, Minoxidil and Tretinoin respectively. Overlay of the three spectra taken and iso-absorptive point was selected and it was found that all three drugs show appreciable absorbance at 243 nm, so it is used for the further study.

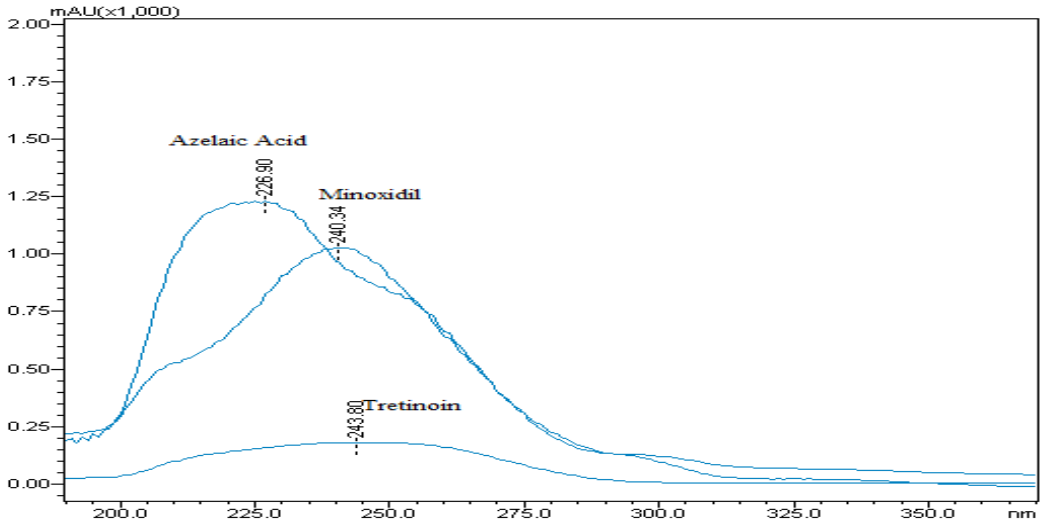

FIGURE 4: Overlay absorption spectrum for Azelaic acid, Minoxidil and Tretinoin

Procedure of Analysis
1ml from Azelaic acid Standard stock solution, 1ml from Minoxidil Standard stock solution and 1ml from Tretinoin Standard stock solution were taken and volume was make up to 10ml with Mobile phase to obtain Working standard solution containing Azelaic acid (15μg/mL), Minoxidil (50μg/mL) and Tretinoin (1μg/mL). 1ml from Sample stock solution was taken into a 10ml volumetric flask, 1ml from Tretenoin stock solution was added and make up with mobile phase to obtain Working sample solution of concentration Azelaic acid (15μg/mL), Minoxidil (50μg/mL) and Tretinoin (1.1μg/mL) respectively.

The contents of standard and sample solution were then filtered through 0.45 µm syringe filter. Chromatograms standard solution (six replicates) was recorded. A typical chromatogram of Azelaic acid, Minoxidil and Tretinoin are presented in figure 5. The retention time of Azelaic acid, Minoxidil and Tretinoin were 3.83 min, 5.83 min and 6.63 min respectively. The peak areas were measured and the quantitation was carried out by keeping these values to the regression equation of calibration curve.

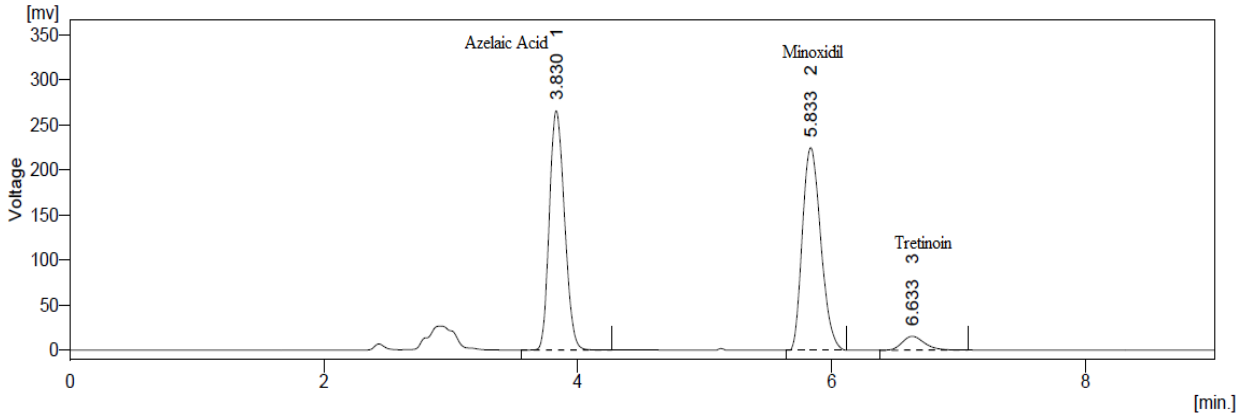

FIGURE 5: Standard Chromatograms of Azelaic acid, Minoxidil and Tretinoin.

Optimized Chromatographic Condition:
Stationary phase: Thermo scientific BDS hypersil C_{18} (250mm × 4.6mm, 5µ).
Mobile phase **:** Potassium dihydrogen phosphate (pH 4.0) : Methanol (80:20)
Flow rate **:** 1.0 ml/min
Run time (min) **:** 8 min
Detection **:** At 243 nm
Injection (volume) **:** 20µl

TABLE 1: System suitability of proposed method

Parameters	Azelaic acid	Minoxidil	Tretinoin
Theoretical plates	4351	7364	6753
Resolution	-	7.947	2.690
Asymmetry	1.290	1.417	1.400
Retention time	3.830 min	5.833 min	6.633 min

Method validation procedure
The developed method was validated for the parameters listed in ICH guidelines [5-8].

Linearity
The method was linear in the range of 7.5-37.515 μg/mL, 25-125 μg/mL and 0.5-2.5 μg/mL for Azelaic acid, Minoxidil and Tretinoin respectively. The linear correlation coefficient for Azelaic acid, Minoxidil and Tretinoin were found to be 0.9995, 0.9999 and 0.9996 respectively, and are recorded in table 2, 3 and 4. Calibration curve of Azelaic acid, Minoxidil and Tretinoin was obtained by plotting the peak area ratio versus the respective concentrations (Figure 6, 7 and 8).

TABLE 2: Linearity results of Azelaic acid

Linearity Level	Concentration	Area
I	7.5 µg/ml	1124.387
II	11.25 µg/ml	1660.257
III	15 µg/ml	2270.922
IV	18.75 µg/ml	2696.014
V	22.5 µg/ml	3411.046
VI	30 µg/ml	4510.859
VII	37.5 µg/ml	5619.787
Correlation coefficient		0.9995

TABLE 3: Linearity of Minoxidil

Linearity Level	Concentration	Area
I	25 µg/ml	1169.123
II	37.5 µg/ml	1725.932
III	50 µg/ml	2359.52
IV	62.5 µg/ml	2905.106
V	75 µg/ml	3530.989
VI	100 µg/ml	4669.557
VII	125 µg/ml	5817.579
Correlation coefficient		0.9999

TABLE 4: Linearity of Tretinoin

Linearity Level	Concentration	Area
I	0.5 µg/ml	85.813
II	0.75 µg/ml	126.119
III	1 µg/ml	162.685
IV	1.25 µg/ml	203.813
V	1.5 µg/ml	254.265
VI	2 µg/ml	332.278
VI	2.5 µg/ml	414.347
Correlation coefficient		0.9996

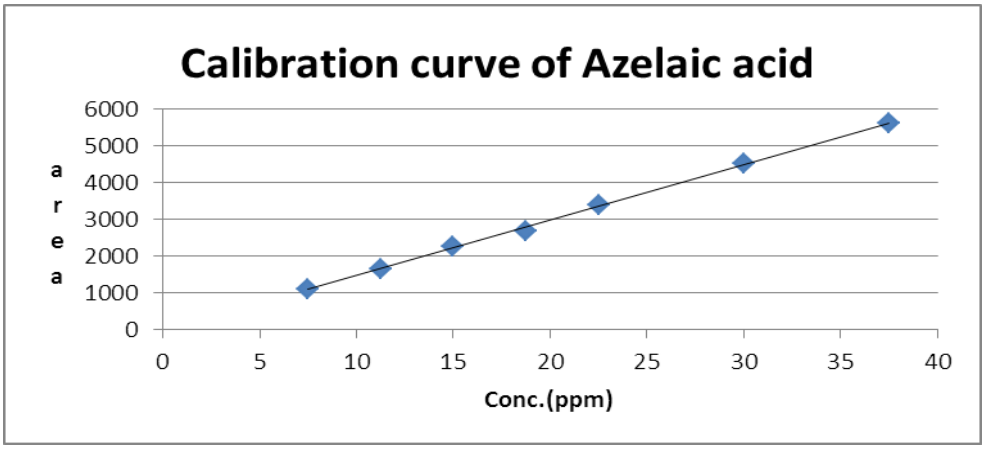

FIGURE 6: Calibration curve of Azelaic acid

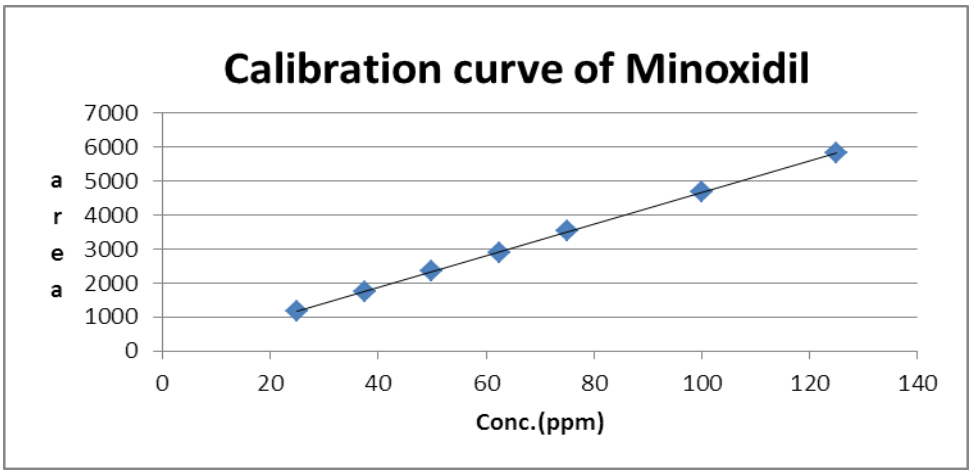

FIGURE 7: Calibration curve of Minoxidil

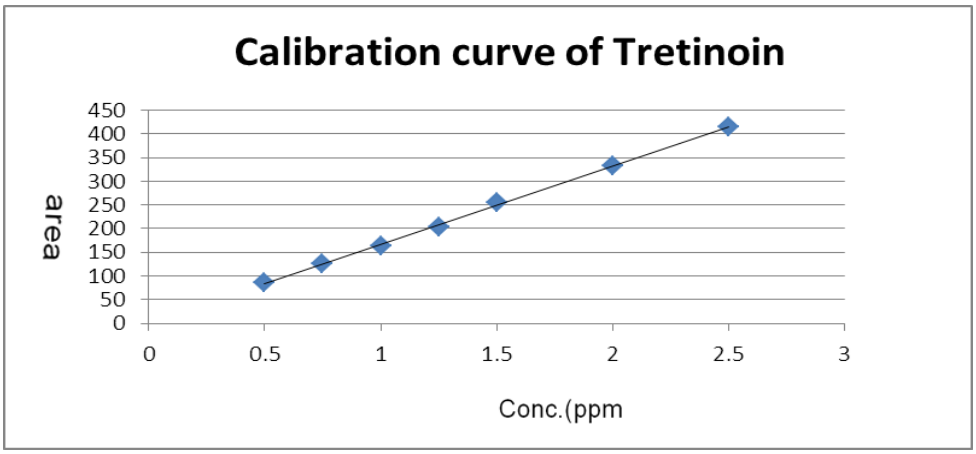

FIGURE 8: Calibration curve of Tretinoin

Accuracy

The accuracy of the method was determined by recovery experiments. Known concentration of working standard was added to the fixed concentration of the pre-analyzed Drop solution. Percent recovery was calculated by comparing the area before and after the addition of working standard. For all the three drugs, recovery was performed in the same way. The recovery studies were performed in triplicate and results are recorded in table 5. This standard addition method was performed at 80%, 100%, 120% level and the percentage recovery was calculated. Percent recovery was within the range of 99.80 to 100.01 for Azelaic acid, 99.53 to 99.60 for Minoxidil and 99.35 to 100.56 for Tretinoin which indicates that the method was accurate.

TABLE 5: Results of Accuracy

Sample	Accuracy	Standard Drug (µg/ml)	Sample Drug (µg/ml)	% of recovery	S.D.	% RSD
Azelaic Acid	80%	12	15	99.80	1.21	1.21
	100%	15	15	100.01	0.74	0.74
	120%	18	15	99.99	0.63	0.63
Minoxidil	80%	40	50	99.53	1.25	1.25
	100%	50	50	99.60	0.89	0.89
	120%	60	50	99.59	0.73	0.73
Tretinoin	80%	0.8	0.1	99.72	1.55	1.55
	100%	1.0	0.1	100.56	0.97	0.96
	120%	1.2	0.1	99.35	1.23	1.23

Precision

For the precision study, repeatability study was carried out for short time interval under the same chromatographic condition. The sample was injected in six replicate. The peak area for all the six replicate was recorded. The mean and % relative standard deviation (%RSD) was calculated and the results are shown in table 6. The %RSD for Azelaic acid, Minoxidil and Tretinoin were found to be 0.79%, 0.75% and 1.27 % respectively. From the data obtained the developed RP-HPLC method was found to be precise. For interday and intraday precision three different concentrations (50%, 100% and 150% of analyte) of standard solutions were injected on same day and three consecutive days in three replicates and results were recorded in table 7 & 8.

TABLE 6: Results of Precision

Injection	Area of Azelaic Acid	Area of Minoxidil	Area of Tretinoin
Injection 1	2313.245	2403.499	165.721
Injection 2	2331.006	2421.93	167.049
Injection 3	2332.036	2423.015	167.096
Injection 4	2364.046	2456.281	169.424
Injection 5	2331.598	2422.571	167.053
Injection 6	2313.428	2410.766	162.998
Average	2330.893	2423.01	166.557
S.D.	18.53	18.10	2.11
% RSD	0.79	0.75	1.27

TABLE 7: Result of Interday Precision

Conc. (µg/ml)			Area			% RSD		
Azelaic acid	Minoxidil	Tretinoin	Azelaic acid	Minoxidil	Tretinoin	Azelaic acid	Minoxidil	Tretinoin
7.5	25	0.5	1116.683	1169.298	85.802	1.03	0.90	0.91
15	50	1	2293.447	2384.811	163.315	0.26	0.33	1.02
22.5	75	1.5	3425.137	3556.861	254.493	0.39	0.13	1.26

TABLE 8: Result of Intraday Precision

Conc. (µg/ml)			Area			% RSD		
Azelaic acid	Minoxidil	Tretinoin	Azelaic acid	Minoxidil	Tretinoin	Azelaic acid	Minoxidil	Tretinoin
7.5	25	0.5	1129.509	1173.297	85.691	0.24	0.40	0.88
15	50	1	2313.915	2409.989	165.575	0.90	0.68	1.03
22.5	75	1.5	3410.889	3549.227	252.694	0.21	0.43	1.61

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

The limit of detection and quantification were calculated using standard deviation of response and slope of the calibration curve and results are recorded table 9. The LOD for Azelaic acid, Minoxidil and Tretinoin was found to be 1.523 µg/ml, 1.718 µg/ml and 0.090 µg/ml respectively. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified. The LOQ for Azelaic acid, Minoxidil and Tretinoin was 4.616 µg/ml, 5.205 µg/ml and 0.273 µg/ml.

TABLE 9: Results of LOD and LOQ

Parameter	Azelaic acid (µg/ml)	Minoxidil (µg/ml)	Tretinoin (µg/ml)
LOD	1.523	1.718	0.090
LOQ	4.616	5.205	0.273

Robustness

Robustness of the method was checked by making slight deliberate changes in chromatographic conditions like flow rate, mobile phase ratio and pH of buffer and the result were recorded in table 10. It was observed that there were no marked changes in chromatograms and % relative standard deviation was found below 2%, which demonstrated that the developed RP-HPLC method is robust.

TABLE 10: Results of Robustness

Condition	Variation	Average Area			% RSD		
		Azelaic Acid	Minoxidil	Tretinoin	Azelaic Acid	Minoxidil	Tretinoin
Flow rate	0.8 min	2201.202	2289.767	156.984	1.72	1.85	1.35
	1.2 min	2365.087	2462.888	170.999	1.37	1.33	1.89
Mobile phase	Buffer: Methanol 82:18	2212.878	2301.282	157.2173	1.43	1.58	0.49
	Buffer: Methanol 78:22	2323.364	2417.629	165.611	0.72	0.54	1.44
pH	4.2	2253.234	2340.362	159.1817	1.33	1.36	0.92
	3.8	2325.092	2420.245	165.4843	0.61	0.92	0.60

Specificity

The specificity of proposed method is justified by the chromatograms of blank, placebo, standard and sample solutions under same chromatographic conditions shown in figure 9. The placebos did not interfere in determination of Azelaic acid, Minoxidil and Tretinoin in commercial topical gel. Specificity of the developed method was also evaluated by applying different stress conditions (oxidation, acid, base, thermal and photolytic) to Azelaic acid, Minoxidil and Tretinoin topical gel.

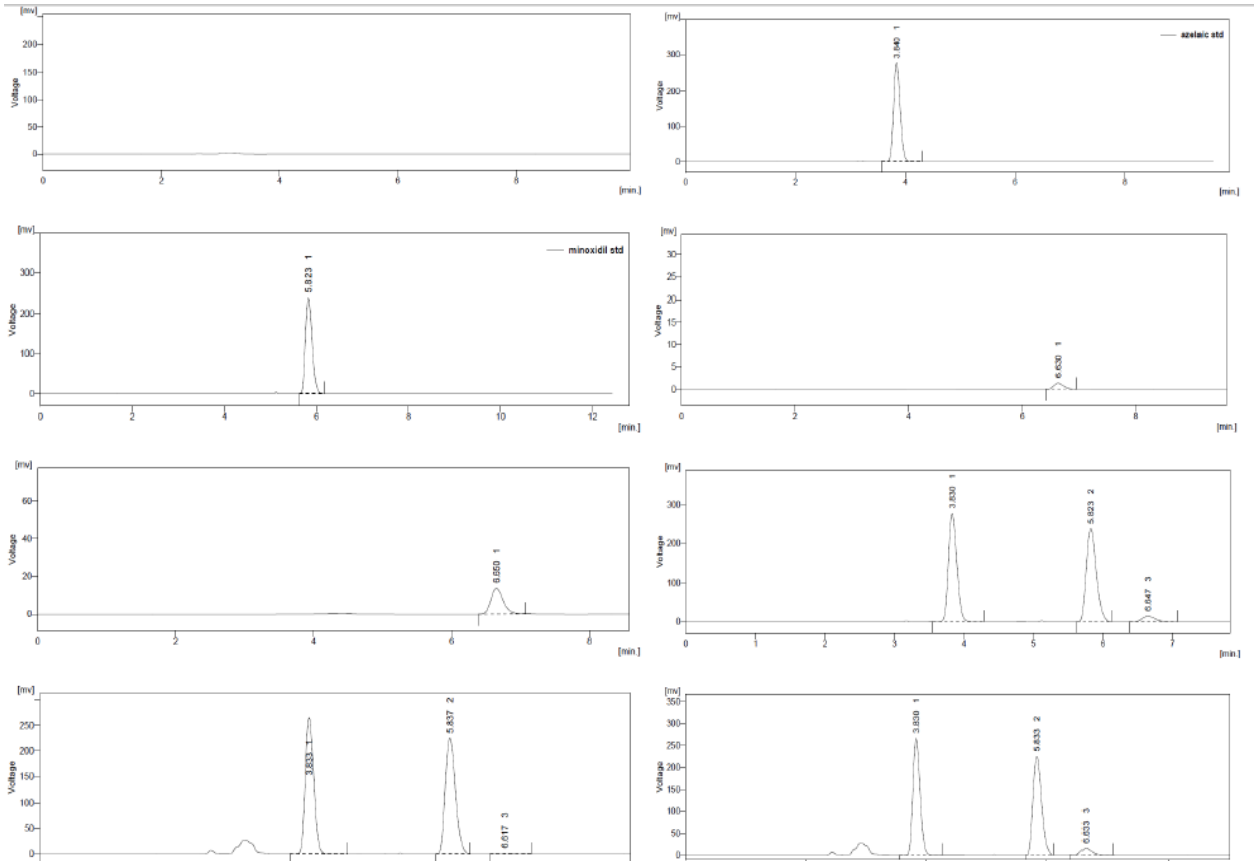

FIGURE 9: Chromatograms of (a) Blank, (b) Azelaic acid, (c) Minoxidil, (d) Tretinoin, (e) Tretinoin with internal standard (1 ppm) (f) Standard mixture, (g) Sample mixture and (h) Sample mixture with Tretinoin internal standard (1 ppm).

Degradation Study

From the results of forced degradation studies showed that these components does not remained intact under stressed conditions and hence special storage conditions should be provided for the dosage form. The specificity studies showed that the principle peaks were well resolved (peak purity 99.99%) and free from any interference from the degradation product. The stress conditions were applied and degraded products of all three drugs are compared and showed in table 10 and chromatograms are in figure 10. From the stress studies it is concluded that substantial degradation of Azelaic acid, Minoxidil and Tretinoin occurred in acid, basic, oxidative thermal and photolytic stress conditions. The degradation products (impurities) in addition to percent degradation under acid, base, oxidation, thermal and photolytic stresses have unique retention times (RT) to acidic stress (8 impurities, RT: 2.357 min, 2.750 min, 3.160 min, 4.477 min, 4.900 min, 5.173 min, 7.193 min and 7.880 min), basic stress (8 impurities, RT: 2.300 min, 2.397 min, 2.797 min, 3.170 min, 4.450 min, 4.867 min, 7.150 min and 7.823 min), oxidative stress (8 impurities, RT: 2.407 min, 2.807 min, 3.163 min, 4.473 min, 4.897 min, 5.133 min, 7.200 min and 7.877 min), thermal stress (5 impurities, RT: 3.130 min, 4.463 min, 4.883 min, 7.250 min and 7.933 min) and photolytic stress (5 impurities, RT: 3.140 min, 4.487 min, 4.910 min, 7.170 min and 7.847 min). Degradation studies justified the method specificity for its intended application.

TABLE 10: Stability study results

Type of degradation	Drug	Peak Area of Standard	Conditions	Peak area			
				Standard		Sample	
				Area	% Deg.	Area	% Deg.
Acid degradation	Azelaic acid	2492.304	4 hours at Room Temperature	1565.199	37.20	1520.842	38.98
	Minoxidil	2825.49		1919.414	32.07	1967.603	30.36
	Tretinoin	164.185		133.749	18.54	130.277	20.65
Base degradation	Azelaic acid	2492.304	2.5 hours at Room Temperature	1758.441	29.45	1785.662	28.35
	Minoxidil	2825.49		1590.17	43.72	1553.683	45.01
	Tretinoin	164.185		125.422	23.61	126.938	22.69
Oxidative	Azelaic acid	2492.304	4 hours at	1699.183	31.82	1723.077	30.86

degradation	Minoxidil	2825.49	Room Temperature	2048.445	27.50	2007.091	28.96
	Tretinoin	164.185		105.235	35.90	104.902	36.11
Thermal degradation	Azelaic acid	2492.304	4 hours at 105^0C	1741.301	30.13	1691.424	32.13
	Minoxidil	2825.49		1782.114	36.93	1808.7	35.99
	Tretinoin	164.185		113.433	30.91	110.964	32.42
Photolytic degradation	Azelaic acid	2492.304	3.5 hours in direct Sun light	1836.963	26.29	1773.389	28.85
	Minoxidil	2825.49		1851.528	34.47	1801.226	36.25
	Tretinoin	164.185		110.634	32.62	110.29	32.83

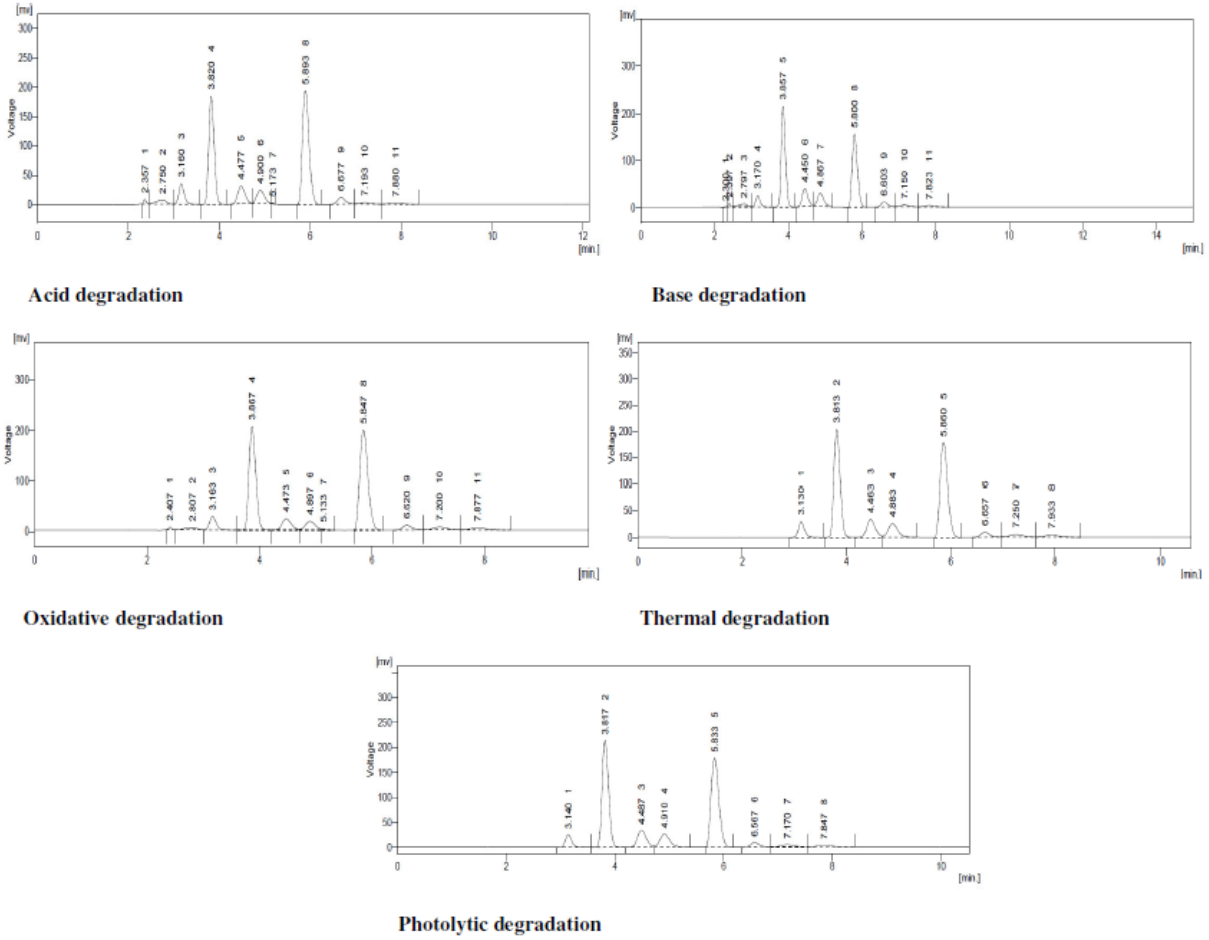

FIGURE 10: Chromatograms of (a) Acid Degradation, (b) Base Degradation (c) Oxidative Degradation, (d) Thermal Degradation, (e) Photolytic Degradation.

RESULTS AND DISCUSSION

To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation and good peak symmetry was obtained with BDS hypersil C_{18}, 250mm×4.6mm internal diameter, 5µ particle size or equivalent column and mobile phase comprising of Buffer (0.05 M potassium dihydrogen phosphate) pH 4.0 : Methanol (80:20v/v) at a flow rate of 1.0 ml/min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 243nm based on peak area. The retention time for Azelaic acid, Minoxidil and Tretinoin were found to be 3.83 min, 5.83 min and 6.63 min, respectively.

The optimized method was validated as per ICH guidelines. The system suitability parameters observed by using this optimized conditions were reported. The method was found to be linear in the concentration range of 7.5-37.5 μg/mL with correlation coefficient of 0.9995 for Azelaic acid, 25–125 μg/mL with correlation coefficient of 0.9999 for Minoxidil and 0.5–2.5 μg/mL with correlation coefficient of 0.9996 for Tretinoin. The results of recovery study (100.01% for Azelaic acid, 99.60% for Minoxidil and 100.56% for Tretinoin) suggest that the method has good recovery. The precision of the proposed method was carried in terms of the repeatability. The low% RSD (<2) values of 0.79%, 0.75% and 1.27% variation for Azelaic acid, Minoxidil and Tretinoin, respectively, reveals that the proposed method is precise. The LOD and LOQ values for Azelaic acid were found to be 1.523µg/ml and 4.616µg/ml, for Minoxidil were 1.718µg/ml and 5.205µg/ml and for Tretinoin were 0.090µg/ml and 0.273µg/ml. The results of robustness in the present method showed no

significant changes. The results of analysis of drop indicated that no interference due to common excipients was observed with the developed method. Degradation studies justified the method specificity for its intended application. Therefore, the proposed method can be used for routine analysis of three drugs in their combined pharmaceutical dosage form.

4. CONCLUSION

A simple, precise, accurate and rapid method was developed for simultaneous estimation of Azelaic acid, Minoxidil and Tretinoin from pure and its dosage forms. The mobile phase is simple to prepare and economical. The sample recoveries in the formulation were in good agreement with their respective label claims. Hence, this method can be easily and conveniently adopted for routine analysis of Azelaic acid, Minoxidil and Tretinoin in pure form and its dosage form.

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