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Stability Indicating Reverse Phase High Performance Liquid Chromatography Method for Simultaneous Estimation of Allantoin, Hydroquinone and Tretenoin in Cream Formulation

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ABSTRACT: In the current study, a reverse phase high performance liquid chromatography (RP-HPLC) approach that was developed and validated for the simultaneous quantification of hydroquinone, allantoin, and tretenoin in a cream formulation is discussed. On a reversed-phase InertsilC_{18} column (4.6 mm I.D. 250 mm, 5 m), the chromatographic separation was completed using a mobile phase made up of buffer (pH 3.5), 0.05 M potassium dihydrogen ortho phosphate-methanol (70:30% V/V), and UV detection at λmax 223nm. The technique demonstrated linearity, with Hydroquinone, Allantoin, and Tretenoin correlation coefficients of 0.999, 0.999, and 0.999 spanning ranges of 100-300 g/mL, 50 to 150 g/mL, and 0.625 to 1.875 g/mL, respectively. All of the components' mean recoveries ranged from 99.00 to 101.00%. The technique was approved in accordance with ICH standards. With no unwelcome interference, the established approach was extremely precise, repeatable, and detectable for hydroquinone, allantoin, and tretenoin together. The approach is effective in isolating the API from its degradants when judged on many criteria, including system suitability, precision, accuracy, linearity, robustness, and stability studies. It can be used to analyse samples of hydroquinone, allantoin, and tretenoin.

Keywords- *Stability Indicating Reverse Phase, High Performance, Liquid Chromatography Method, Simultaneous Estimation, Allantoin, Hydroquinone, Tretenoin, Cream Formulation.*

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INTRODUCTION

A combine cream formulation of hydroquinone (2%), allantoin (1%) and tretenoin (0.012%) is available as cream formulation used in the treatment of Melasma disease.[1] Melasma is a common skin problem. The condition causes dark, discoloured patches on your skin. Hydroquinone chemically benzene-1, 4-diol or quinol is an phenol type aromatic organic compound derived from benzene, having the chemical formula C6H4 (OH)2 (Fig. 1). Hydroquinone is a topical depigmenting agents agent used in hyper pigmentation conditions.[2] It produces lightening of your skin by decreasing the number of melanocytes present or produced in your skin.[3] Allantoin is 2, 5-dioxo-4-imidazolidinyl-urea Allantoin is a skin protectant which works as a moisturizer and relieves minor skin irritations.[4] Allantoin is also known as kerolytic molecules that remove warts, corns and horny layer of the skin. Tretenoin is a form of vitamin A that helps the skin to renew itself more quickly. It is used to treat Acne or skin disease such as wrinkles, dark spot and rough skin.
Hydroquinone and Allantoin is an official drug in USP while Tretenoin are official in both USP and BP. Literature revealed some analytical methods for the analysis of HQ either alone or in various combination[6,7] including spectrophotometry[8] and high performance liquid chromatography (HPLC).[9-14] Literature study reveals that no stability indicating reversed phase-high

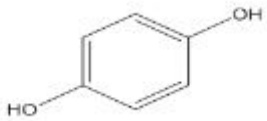


Fig. 1- Structure of Hydroquinone

performance liquid chromatographic (RP-HPLC) methods are available for the estimation of hydroquinone, allantoin and Tretenoin combine formulation. The aim of this present study was to develop and validate a stability indicating RP-HPLC method for simultaneous estimation of allantoin, hydroquinone and tretenoin in cream formulation. The stability studies of pharmaceutical products are one of the very important parameter for development of new drugs as well as new formulations. The shelf-life prediction is a major role for the pharmaceutical product development of all the dosage forms and also it is utilized to determine the particular storage conditions and to suggest label instructions. Stability studies of pharmaceutical products ensuring the maintenance of product quality, safety and efficacy throughout the shelf life are considered as pre-requisite for the acceptance and approval of any pharmaceutical products [5].

MATERIAL AND METHOD

(i) Chemicals and Reagents-Pharmaceutically pure samples of hydroquinone, allantoin and tretenoin were obtained as a gift sample from R.K. School of Pharmacy, Loba Chemical Private limited, Mumbai, and Abbott Pharmaceutical, Mumbai respectively. Acetonitrile and Methanol were obtained from Merck Specialties Private Limited, Mumbai and Molychem, Mumbai, respectively. HPLC grade water was obtained from Loba Chemie Pvt. Ltd., Mumbai and Astron Chemicals, Ahmadabad respectively.
(ii) Instrumentation-HPLC instrument having UV-Visible detector, Shimadzu: the separation was performed on InertsilC18 (4.6 mm I.D. × 250 mm, 5 μm).
(iii) Buffer Preparation (0.05M potassium dihydrogenortho phosphate, pH-3.5)-6.8 gm potassium dihydrogen ortho phosphate reagent was taken into 1000 mL volumetric flask.

Then 800 mL water was added to dissolve and pH 3.5 was adjusted with 1% Orthophosphoric acid. Finally, volume was made up to 1000 mL with water.

(iv) Preparation of Standard Solution-Accurately weighted quantity of hydroquinone 200 mg, allantoin 100 mg and tretenoin 12.5 mg was transferred into different 100 ml volumetric flask to get concentration of hydroquinone 2000 µg/mL, allantoin 1000 µg/mL, tretenoin 125 µg/mL.

(v) Preparation of Combined Working Standard Solution-1-mL from hydroquinone, allantoin and tretenoin standard stock solution were taken in 10 mL volumetric flask and finally volume made up to 10 mL with mobile phase to get concentration hydroquinone 200 µg/mL, allantoin 100 µg/ mL, tretenoin 1.25 µg/mL.

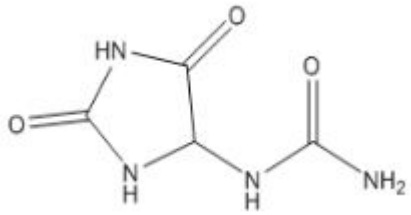


Fig. 2- Structure of Allantoin

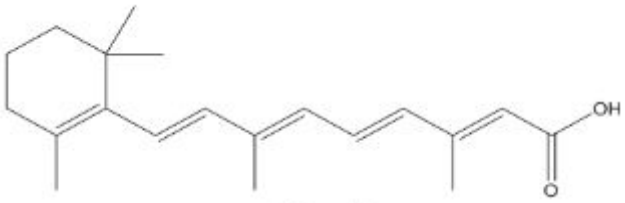


Fig. 3- Structure of Tretenoin

METHOD VALIDATION

Method was validated as per ICH guidelines.

(i) System Suitability-The system suitability was assessed by triplicate analyses of the drugs at a concentration of 200, 100, 1.25 µg/mL of hydroquinone, allantoin and tretinoin, respectively. System suitability parameters were shown in Table 2.

Parameter	*Result*
Mobile Phase	Buffer 0.05M potassium dihydrogen ortho phosphate (pH 3.5) Methanol in the ratio of 70:30 %V/V
Column	$Inertsilc_{18}$
Flow Rate	1-mL/min
Wave length	223 nm
Injection Volume	20 µL
Retention Time	Hydroquinone: 3.9 min Allantoin: 6.3 min Tretenoin: 8.6 min
Run Time	10 minutes

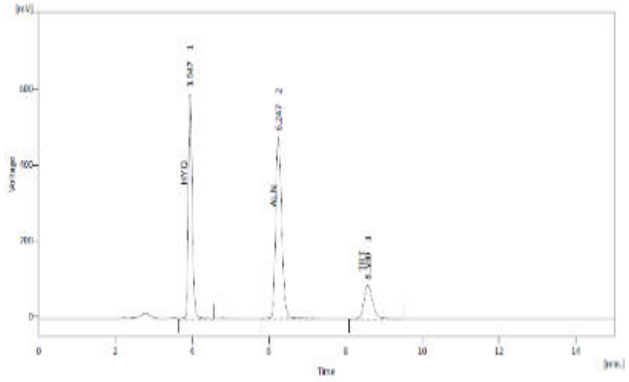
Table 1- Optimised chromatographic conditions

Retention Time	*Area*	*Height*	*Asymmetry*	*Efficiency*	*Resolution*
3.960	4129.144	593.185	1.346	7180	-
6.330	5678.481	470.835	1.419	6371	9.402
8.627	2138.884	140.765	1.382	7158	6.335

Table 2- System suitability parameter

Hydroquinone (2%)		*Allantoin (1%)*		*Tretenoin (0.0125%)*	
Conc. (µg/mL)	*Area*	*Conc. (µg/mL)*	*Area*	*Conc. (µg/mL)*	*Area*
100	2052.146	50	2822.120	0.625	1061.556
150	3089.538	75	4248.805	0.937	1600.348
200	4132.373	100	5682.945	1.250	2140.578
250	5148.993	125	7089.343	1.562	2667.745
300	6128.679	150	8438.344	1.875	3175.369

Table 3- Linearity data



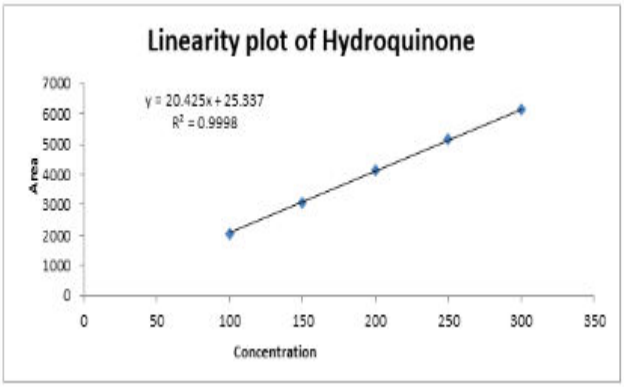
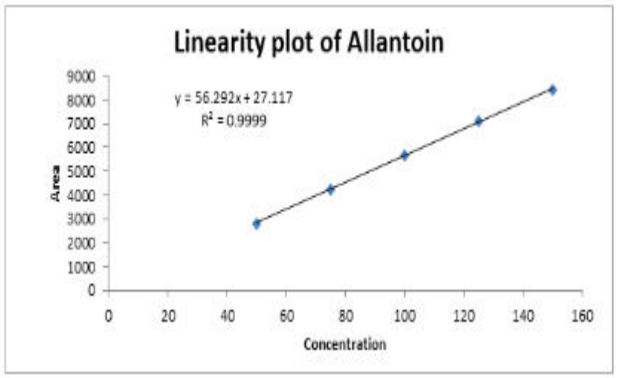


Fig. 4- Chromatogram of sample solution **Fig. 5- Calibration curve hydroquinone X-axis: Concentration Y-axis: Area**



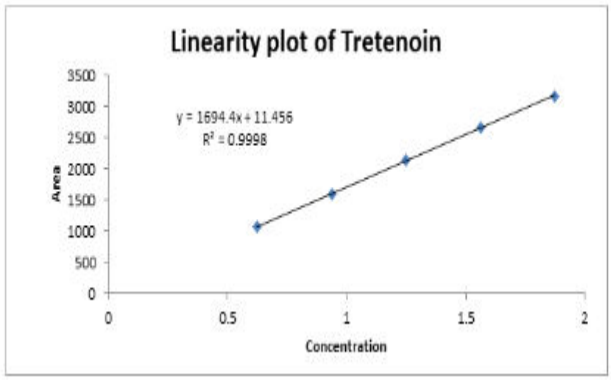


Fig. 6- Calibration curve allantoin X-axis: Concentration Y-axis: Area
Fig. 7: Calibration curve tretenoin X-axis: Concentration Y-axis: Area

(ii) Specificity-At the retention time of 3.9, 6.3 and 8.6 min, the proposed method was specific for the detection of hydroquinone, allantoin, tretinoin, respectively. There were no peaks at the retention time of hydroquinone, hydrocortisone, tretnoin.
(iii) Linearity-Linearity was tested in the concentration range µg/mL for 100, 150, 200, 250, 300 for hydroquinone 50, 75, 100, 125, 150 for allantoin 0.62, 0.93, 1.25, 1.56, and 1.87 for tretinoin. All the solutions were measured six times in accordance with the ICH. The typical table of linearity are shown in Table 3 and calibration curve shown in Figs. 5 to 6.
(iv) Precision-For repeatability standard solution containing hydroqui- none (200 µg/mL), allantoin (100 µg/mL) and tretinoin (1.25 µg/mL) were injected six times and area of peak were measured. For intraday precision, the solutions were analyzed six times on the same day and for interday precision, the solutions were analyzed six times on the different day and % RSD was calculated. Precision condition was shown in Table 4.
(v) Accuracy-Recovery studies were carried out by applying the method to drug sample present in topical dosage form to which known amount of hydroquinone, allantoin and tretenoin
corresponding to 80, 100, and 120% of label claim was added by standard addition method. Accuracy condition was shown in Tables 5 to 7.

	Drug	*Concentration*	*Peak Area ± SD*	*%RSD*
Repeatability	Hydroquinone	200	4165.95 ± 0.773	0.769
	Allantoin	100	5691.95 ± 0.670	0.670
	Tretenoin	1.25	2095.78 ± 0.547	0.556
Intraday Precision	Hydroquinone	200	4127.63 ± 0.731	0.726
	Allantoin	100	5690.33 ± 0.601	0.600
	Tretenoin	1.25	2108.37 ± 0.560	0.560
Interday Precision	Hydroquinone	200	4161.66 ± 0.810	0.810
	Allantoin	100	5670.71 ± 0.660	0.660
	Tretenoin	1.25	2078.52 ± 0.560	0.510

Table 4- Precision

Amt of hydroquinone present in sample (µg/mL)	*Amt of std hydroquinone added (µg/mL)*	*Total amount found (µg/mL)*	*%Recovery*	*Mean % recovery*	*SD*	*% RSD*
100	80	79.398	99.247	99.351	0.763	0.768
100	80	78.917	98.646			
100	80	80.129	100.161			
100	100	99.662	99.662	99.375	0.975	0.981
100	100	100.170	100.170			
100	100	98.286	98.286			
100	120	119.446	99.539	99.087	1.132	1.143
100	120	117.358	97.798			
100	120	119.907	99.923			

Table 5- Accuracy data for Hydroquinone

Amt of Allantoin present in sample (µg/mL)	*Amt of std allantoin added (µg/mL)*	*Total amount found (µg/mL)*	*% recovery*	*Mean % recovery*	*SD*	*% RSD*
50	40	39.710	99.275	99.322	0.901	0.908
50	40	39.378	98.445			
50	40	40.099	100.247			
50	50	49.839	99.678	99.585	0.722	0.725
50	50	50.128	100.256			
50	50	49.410	98.820			
50	60	59.748	99.581	99.230	0.942	0.949
50	60	58.898	98.163			
50	60	59.968	99.946			

Table 6- Accuracy data for Allantoin

Amt of tretenoin present in sample (µg/mL)	*Amt of std tretenoin added (µg/mL)*	*Total amount found (mean) (µg/mL)*	*% recovery*	*Mean % recovery*	*SD*	*% RSD*
0.625	0.5	0.498	99.543	99.452	1.096	1.102
0.625	0.5	0.492	98.313			
0.625	0.5	0.503	100.500			
0.625	0.625	0.624	99.919	99.563	1.132	1.136
0.625	0.625	0.628	100.474			
0.625	0.625	0.614	98.297			
0.625	0.75	0.748	99.794	99.511	0.832	0.836
0.625	0.75	0.739	98.574			
0.625	0.75	0.751	100.164			

Table 7- Accuracy data for tretenoin

(vi) Limits of Detection and Quantification (LoD and LoQ)- The LoD values were found to be 3.75, 1.80, 0.02 for hydroquinone, allantoin and tretinoin, respectively. The LoQ values were found to be 11.37, 5.44 and 0.07 for hydroquinone, allantoin and tretinoin, respectively. LoD and LoQ condition are shown in Table 8.

(vii) Robustness- It was measured by changing pH, Ratio of mobile phase and flow rate. The pH of mobile phase was set at ± 0.2, Ratio of Mobile phase was set ± 5 mL and flow rate was set at ± 0.2 mL/min. Solution of both the drugs was injected three times. Robustness condition was shown in Table 9.

ANALYSIS OF MARKETED FORMULATION

Weight about 10 gm topical cream into a 100 mL 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 up to 100 mL. Filter this solution with what man filter paper no-1. So finally became hydroquinone-2000 µg/mL, allantoin-1000 µg/mL, and tretenoin-12.5 µg/mL. The results are shown in Table 10.

(i) Force Degradation Studies-The drugs were intentionally degraded by treating with Acid, Base, Thermal, Oxidation and exposing to Sunlight condition. Degradation condition shown in Table 11 and Figs. 9–13.

(ii) Acid Degradation-For Acid Degradation for Blank, 2 mL 0.1N HCl and 2 mL 0.1N NaOH was taken and volume made up the volume 10 mL with mobile phase. For hydroquinone, allantoin and tretenoin standard degradation, 1mL hydroquinone, allantoin and tretenoin stock solution was taken and 2 mL 0.1N HCl was added then kept for 5 hours and neutralize with 2 mL 0.1N NaOH to stop the degradation further. Finally, volume was made up to 10mL with mobile phase. For sample degradation also same procedure was followed.

1. Parameters	2. Allantoin	3. Hydroquinone	4. Tretenoin
5. Linearity range (µg/mL)	6. 100–300 µg/mL	7. 50–150 µg/mL	8. 0.6–1.8 µg/mL
9. Correlation Co-efficient	10. 0.999	11. 0.999	12. 0.999
13. Slope Mean	14. 20.43	15. 56.29	16. 1694.41
17. SD of Intercept	18. 23.23	19. 30.64	20. 12.26
21. LOD	22. 3.75	23. 1.80	24. 0.02
25. LOQ	26. 11.37	27. 5.44	28. 0.07

Table 8- LOD and LOQ

Change in flow rate	*Buffer(pH4) Methanol /80:20*				*pH 4*	
Hydroquinone						
Level	Avg. Area ± SD	% RSD	Avg. Area ± SD	% RSD	Avg. Area ± SD	% RSD
+0.2	3920.75 ± 34.90	0.890	3908.60 ± 40.01	1.024	4107.04 ± 30.65	0.746
-0.2	4299.36 ± 37.19	0.865	4299.47 ± 43.93	1.022	4112.39 ± 42.16	1.025
Allantoin						
+0.2	5398.35 ± 40.79	0.756	5383.82 ± 40.41	0.751	5653.99 ± 51.17	0.905
-0.2	5901.31 ± 64.18	1.088	5924.16 ± 43.01	0.726	5653.44 ± 61.02	1.079
Tretenoin						
+0.2	2025.37 ± 20.79	1.026	2025.02 ± 20.18	0.997	2125.92 ± 25.72	1.210
-0.2	2221.20 ± 26.30	1.184	2225.33 ± 25.58	1.150	2128.26 ± 24.70	1.161

Table 9- Robustness Data

Drug	*Label claim (%w/w))*	*Result (% w/w)*	*%Assay*	*Mean %Assay*	*%RSD*
Hydroquinone	2	2.029	101.437	100.639	0.872
	2	1.994	99.698		
	2	2.016	100.780		
Allantoin	1	1.008	100.768	99.959	0.991
	1	0.989	98.854		
	1	1.003	100.254		
Tretenoin	0.0125	0.012	99.959	97.791	0.744
	0.0125	0.012	97.059		
	0.0125	0.012	97.798		

Table 10- Analysis of marketed formulation

	Hydroquinone	*Allantoin*	*Tretenoin*
Condition	*% Drug degradation*		
Acid	22.74	17.46	10.50
Base	14.17	11.91	20.45
Oxidation	12.28	25.57	18.66
Photo	16.36	20.25	8.82
Thermal	10.99	13.89	10.12

Table 11- Result of degradation study

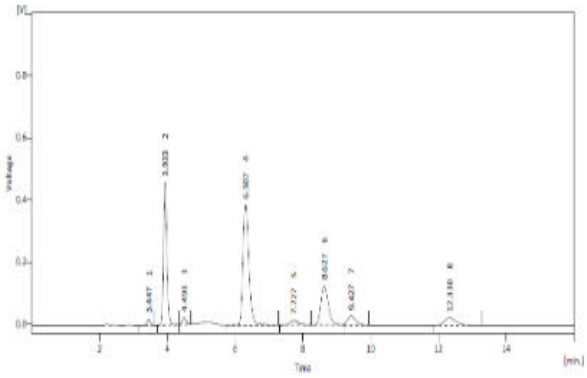


Fig. 8- Acid degradation study

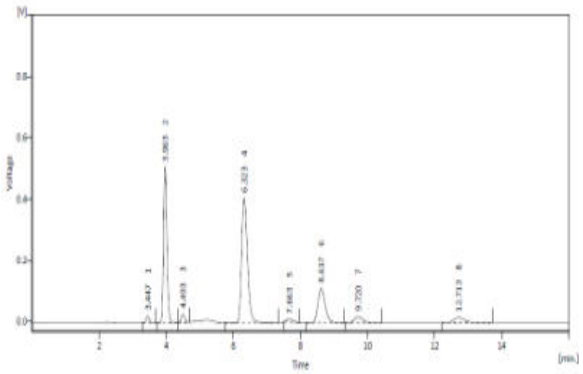


Fig. 9- Base degradation study

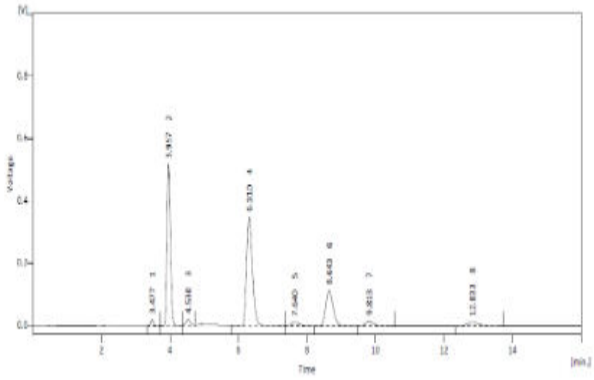


Fig. 10- Oxidative degradation study

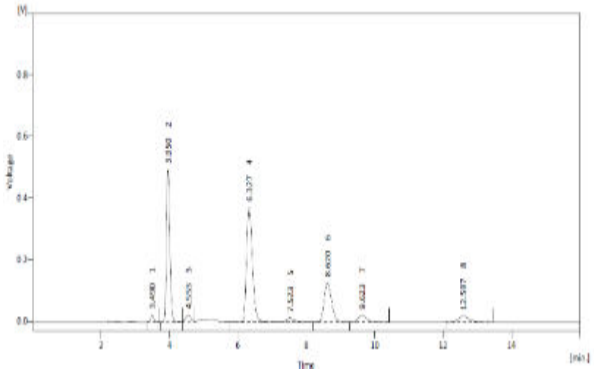


Fig. 11- Photo degradation study

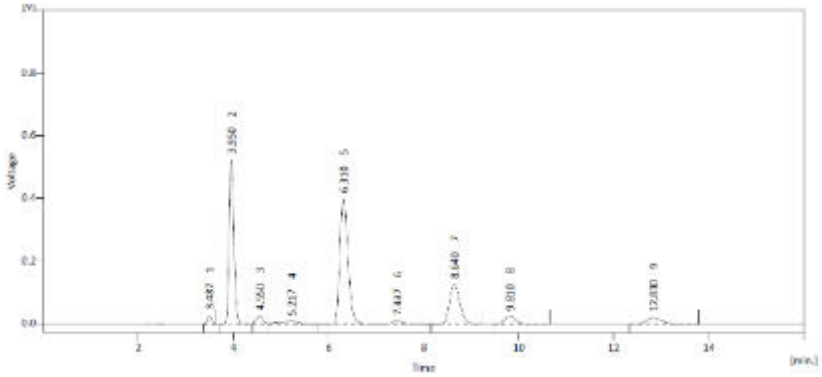


Fig. 12- Thermal degradation study

(iii) Base Degradation-For Base Degradation blank preparation, 2 mL 0.1 N NaOH and 2 mL 0.1 N HCl was taken and volume makes up to 10 mL with mobile phase. For standard degradation,
hydroquinone, allantoin and tretenoin were taken 1-mL from stock solution and 2 mL of 0.1N NaOH was added then kept for 3.5 hours and neutralize with 2 mL 0.1N HCl to stop the degradation further. Finally, volume was made up to 10 mL with mobile phase. For sample degradation also same procedure was followed.
(iv) Oxidation Degradation-For Oxidative Degradation blank preparation 2 mL of 3% H2O2 was taken and volume made up to 10 mL with mobile Phase. For hydroquinone, allantoin and tretenoin, 1-mL stock solution was taken and 3% H2O2 was added and kept for 3 hours. Finally volume was made up to 10mL with mobile phase. For sample degradation also same procedure was followed.
(v) Thermal Degradation-For Thermal Degradation blank preparation, 2 mL mobile phase was kept at 105^0C and then volume made up to 10 mL with mobile phase. For hydroquinone, allantoin and
tretenoin, 1-mL stock solution were taken and kept at 105^0C for 4.5 hours. Finally, volume was made up to 10mL with mobile phase. For sample degradation also same procedure was followed.
(vi) Sunlight Degradation-For Sunlight Degradation, 1-mL hydroquinone, allantoin and tretenoin were taken and kept at sunlight for 3.5 hours. Finally, volume was made up to 10 mL with mobile phase. For sample degradation also same procedure was followed.

CONCLUSIONS

The findings of our investigation show that the suggested RP-HPLC stability indicating method is easy, quick, precise, and accurate. For the routine examination of hydroquinone, allantoin, and tretinoin in cream topical formulation, this method was created and validated. According to the outcome, the suggested approach might be successfully used for routine analysis and quality control of pharmaceutical dosage forms comprising tretinoin, allantoin, and hydroquinone. The excipients and solvent were kept out of the mobile phase conditions, which were optimised. The mobile phase was chosen to comprise Buffer (pH 3.5) 0.05M Potassium Dihydrogen Orthophosphate-Methanol in a ratio of 70:30% V/V. Allantoin and tretenoin solutions in methanol were scanned between 200 and 400 nm to find the best wavelength for simultaneous quantification of hydroquinone. It was determined from the overlay UV spectra that 223 nm was the best wavelength for examination of all the medicines with adequate sensitivity. The procedure is reproducible and selective for the analysis of this cream formulation, according to statistical analysis. Because there was less deterioration

shown when the medicine was stressed under expedited circumstances, the Method was discovered to be stable. Therefore, it can be concluded that using the technology can result in significant time and financial savings and that it can be used with high precision and a broad linear range in small laboratories.

REFERENCES

[1]. Bandyopadhyay D. Topical treatment of melasma. Indian journal of dermatology. 2009; 54(4):303-9.

[2]. Godse KV. Triple combination of hydroquinone, tretinoin and mometasone furoate with glycolic acid peels in melasma. Indian journal of dermatology. 2009 Jan; 54(1):92.

[3]. The United States Pharmacopoeia 35 and the National Formulary 30: The United States Pharmacopeia Convention. Rockville, MD, USA, 2007.

[4]. The British Pharmacopoeia. Her Magesty's Stationary Office, London, (2013); Electronic version.

[5]. Aashigari S, Goud R, Sneha S, Vykuntam U, Potnuri NR. Stability studies of pharmaceutical products. World Journal of Pharmaceutical Research. 2018; 8(1):479-92.

[6]. Poongothai G, Sripathi S K. Quantification Analysis of Allantoin in leaves, stem and roots of pisonia grandis R.Br by RP-HPLC.2013;5(8):2105-2108.

[7]. Shilu R, Kapupara P. A Stability Indicating Reverse Phase High Performance Liquid Chromatography Method for Simultaneous Estimation of Hydroquinone Hydrocortisone and Tretenoin in Cream Formulation. Journal of Pharmaceutical Research International.2021; 33(39B): 67-78.

[8]. Jogarami R, Jain P, Sharma S. Validated UV spectrophotometric method development for simultaneous estimation of tazarotene and hydroquinone in gel preparation. Journal of Pharmacy Research.2012;5(4):2273-5.

[9]. Kinjal S, Ketan S, Pankaj K.Development and Validation of Analytical Method for Simultaneous Estimation of Mometasone Furoate, Hydroquinone and Tretenoin in Topical Formulation by RP-HPLC. Journal Chem Pharma Res. 2014; 6(4):934-940.

[10]. Harikishor B, Suhail A. Devlopment of analytical method for simultaneous estimation of hydroquinone and Avobenzene in Pharmace-Utical Cream Formulation by RP-HPLC. International
Journal of Pharmacy and Research.2017;10(1):320-332

[11]. Mustafa AM, Aziz ME. Determination of Hydroquinone in Skin Lightening Creams Sold in Sudan by Using High Performance Liquid Chromatography. The Journal of Middle East and North Africa Sciences. 2016 Jul;10(3924):1-5.

[12]. El Mubarak M A, Lamari FN, Kontoyannis C . Simult aneous determination of allantoin and glycolic acid in snail mucus and cosmetic creams with high performance liquid chromatography
and ultraviolet detection. Journal of Chromatography A. 2013 Dec 27;1322:49-53.

[13]. George SK, Dipu MT, Mehra UR, Singh P, Verma AK, Ramgaokar JS. Improved HPLC method for the simultaneous determination of allantoin, uric acid and creatinine in cattle urine. Journal of Chromatography B. 2006 Feb 17;832(1):134-7.

[14]. Chinmoy R, Jitamanyu C, Stablity Indicating RP-HPLC Method Development And Validation for Determination of Potential Degradation Impurities of Tretenoin in topical Pharmaceutical Formulation. Pelagia Research Library, Der Pharmacia Sinica. 2013; 4(4):6-14.

[15]. ICH. Q2R1, Guideline on Validation of Analytical Procedures, Text and Methodology.2005.