INTERNATIONAL JOURNAL OF DRUG DISCOVERY AND HERBAL RESEARCH (IJDDHR)
13(3):Oct.-Dec: (2023), 62-72
ISSN: 2231-6078

Research Article

METHOD DEVELOPMENT FOR ASSAY OF HYDROQUINONE, TRIAMCINOLONE ACETONIDE AND TRETINOIN

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Abstract

Objective of the present work is to develop method for assay of hydroquinone, triamcinolone acetonide and tretinoin. Estimated by reverse phase HPLC using acetonitrile; tetrahydrofuran and sodium acetate buffer pH 4.5 adjusted with glacial acetic acid and column LichroCART (250*4.0 mm, 5um) as a stationary phase. After development of the method it was validated for specificity, linearity, precision, accuracy Studies. Precision RSD was found to be 0.821, 0.254, and 0.851 for hydroquinone, triamcinolone acetonide and tretinoin. This indicates that the method is precise and accurate. The accuracy table it was found that recovery value of pure drug from the reanalyzed solution of formulation were between 98.0 % to 102% which indicates that the method is accurate and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods. Theoritical plate for hydroquinone was found to be not less than 2500 , for triamcinolone acetonide it was to be about 250000 and for tretinoin it was found to be not less than 65000. The sample recoveries in all the formulation were in good agreement with their respective label claim and their suggestive not interference of formulation of excipients in the estimation. Hence this method can be easily and conveniently adopted for routine analysis of hydroquinone, triamcinolone acetonide and tretinoin in bulks and the pharmaceutical dosage forms and also for stability analysis.

Keywords: Method Development, hydroquinone, triamcinolone acetonide, tretinoin, assay, validation

Introduction

Analytical chemistry is a branch of chemistry that determines the nature and identity of a substance and its composition[1]. In the early twentieth century there were only four accepted branches of chemistry, organic chemistry, inorganic chemistry, physical chemistry and biochemistry[2]. At that time, analysis was considered to be a service to the other four branches. Its importance grew, and in the process, absorbed techniques and skills from all other four branches so by the 1950s, analytical chemistry was finally accepted as a branch of chemistry in it own right. There are basically two types of analysis, qualitative analysis and quantitative analysis[3]. The former identifies the nature of substance, and if it is mixture, the nature of the components present, whereas, the latter determines the elemental composition of the substance and/or the quantitative distribution of each component[4]. Most analytical procedures start with some type of separation process, filtraton
distillation, extraction, and centrifugation and, what is most likely today, some form of chromatography[5]. Chromatography, in any one of its different forms, is probably the most important technique available to the analyst[6].

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Chromatography not only separates a mixture into its constituents, but also provides assistance in their identification and gives a quantitative estimation of the amount of each constituent present in the mixture[7]. Any analytical laboratory devoid of any chromatographic technique would, indeed, be restricted in its scope and performance[8]. The techniques of the analytical chemist are of vital importance to the drug and pharmaceutical industries[9]. The products are usually complex organic compounds or mixtures[10]. Drugs prepared for human consumption requires that strict standards of product quality be established and maintained[11].

Materials and Methods[6-13]

Hydroquinone obtained from Symbiotec Pharma Lab Ltd., Triamcinolone acetonide from Eastman Phrama and Tretinoin from Nicholas Piramal. Acetonitrile, Methanol, Glacial Acetic acid and water were belongs to HPLC grade purchased from Merk and Sodium acetate trihydrate belongs to A. R. grade.

Method Development for assay of hydroquinone, triamcinolone acetonide and tretinoin

Number of trials were performed to obtained optimized method for simultaneous estimation of hydroquinone, triamcinolone acetonide and tretinoin

Table No. 1: Chromatographic Conditions for trial 1 & 2

Parameter / conditions	Description/ Values	
	Conditions 1	Conditions 2
Column name	Lichro CART Lichro sphere 250*4.0mm , 5um	INERTSIL ODS 250*4.6mm , 5um
Detector	254nm	254nm
Flow rate	1.0ml/min	1.0ml/min
Injection volume	20ul	20ul
Temperature	Ambient	Ambient
Run time	60min	60min
Mobile phase	Buffer 0.05N :Acetonitrile:Tetrahydrofuran (320:650:30)	Buffer 0.001N :Acetonitrile:Tetrahydrofuran (320:650:30)
Observation	No resolution found Rt of hydroquinone was 2.14 min i.e. obtained before 3 min.	No resolution found Rt of hydroquinone was 2.35 min i.e. obtained before 3 min.

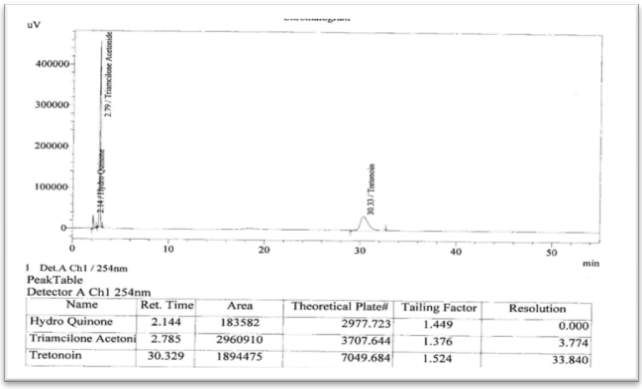


Figure 1: HPLC graph for Chromatographic conditions

Preparation of Buffer (Solvent A): Weighted and dissolved 6.8 gm of sodium acetate trihydrate in 1000 ml of water. The pH 4.5was adjusted with glacial acetic acid. Filtered the solution through 0.45 um membrane filters.

Preparation of Solvent mixture (Solvent B): Mixed Acetonitrile 1000ml and 50 ml of Tetrahydrofuran. Both mixture A and B was Sonicated in ultrasonic bath for 15 to 20 minutes.

Table No. 2: Chromatographic Conditions for Trial 3 & 4

Parameter/conditions	Description/Values
Column name	LichroCARTLichrosphere 250*4.0mm , 5um
Detector	270nm
Flow rate	1.0ml/min
Injection volume	20ul
Temperature	Ambient

Tab. No. 3 Gradient program used Conditions for Trial 3 & 4

Gradient program for Trial -3			Gradient program Trial -4		
Time	**Solvent A**	**Solvent B**	**Time**	**Solvent A**	**Solvent B**
0.01	95	5	0.01	95	5
7	95	5	15	90	10
15	10	90	25	60	90
25	10	90	40	90	10
30	95	5	50	95	5
35	95	5	60	95	5
Peak purity of the triamcinolone acetonide was failed			Only hydroquinone and triamcinolone acetonide peaks were determined with Rt 6.8 min and 26.2 min respectively. No peak of tretinoin was found.		

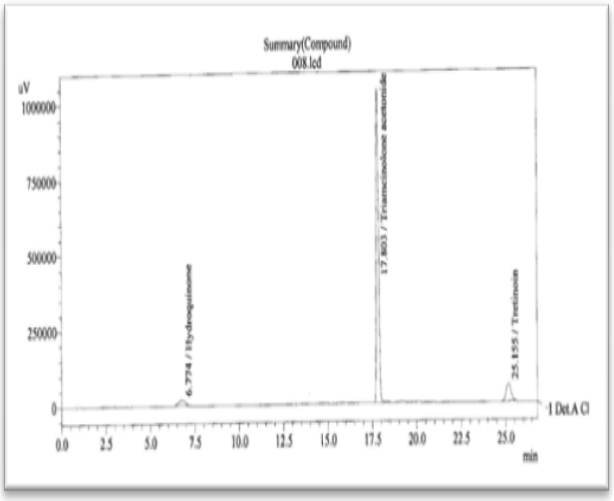


Figure 2: HPLC graph for Chromatographic conditions 3

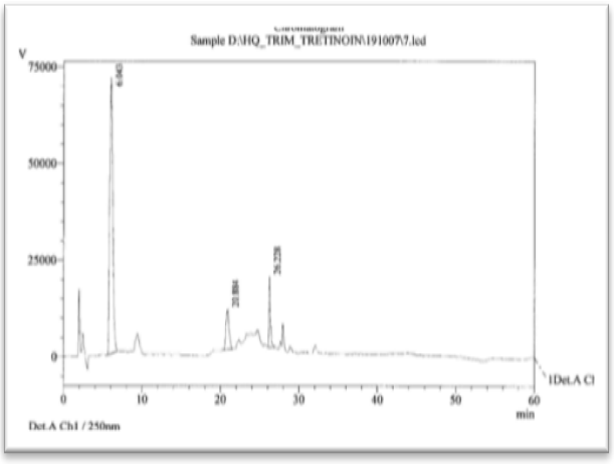


Figure 3: HPLC graph for Chromatographic conditions 4

Table 4: Chromatographic Conditions for Trial 5 & 6

Parameter/ conditions	Description/Values
Column name	LichroCARTLichrosphere 250*4.0 mm , 5um
Detector	250nm
Flow rate	1.0ml/min
Injection volume	20ul
Temperature	Ambient

Table No. 5: Gradient program used Conditions for Trial 5 & 6

Gradient program for Trial -5			Gradient program Trial -6		
Time	**Solvent A**	**Solvent B**	**Time**	**Solvent A**	**Solvent B**
0.01	90	10	0.01	90	10
12	90	10	10	90	10
35	25	75	25	25	75
50	25	75	40	25	75
55	90	10	45	90	10
60	90	10	50	90	10
No good peaks obtained			Good resolution was obtained. All the three compounds were passed		

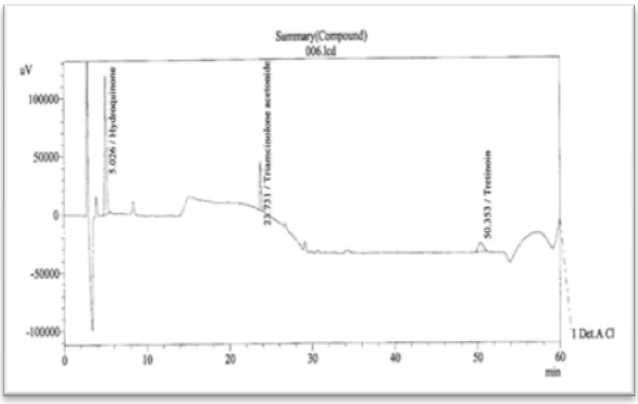


Figure 4: HPLC graph for Chromatographic conditions 5

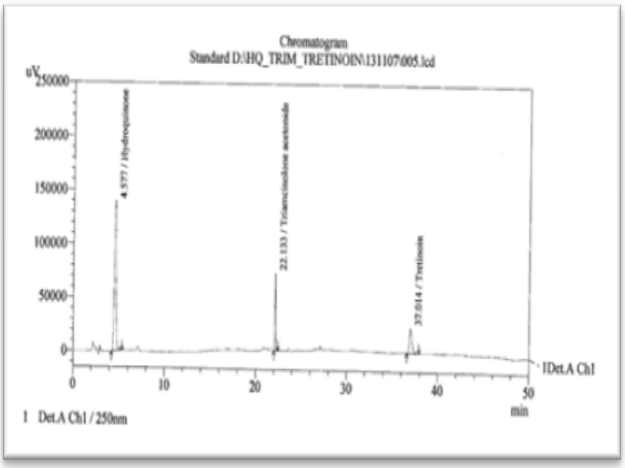


Figure 5: HPLC graph for Chromatographic conditions 6

VALIDATION OF THE DEVELOPED RP-HPLC METHOD

The developed method was validated as per ICH guidelines:

(A) Precision

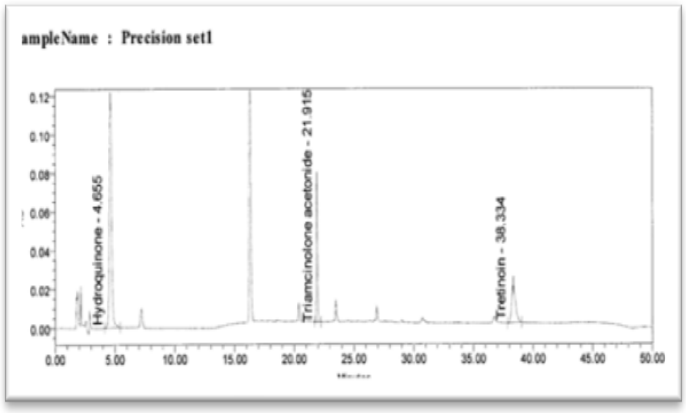


Figure 7: HPLC graph for Sample solution for hydroquinone , triamcinolone acetonide and tretinoin

(B) Linearity:

Solution of the concentration level 50, 80, 90, 100, 110, 120 and 150 % of the hydroquinone, triamcinolone acetonide and tretinoin was prepared.

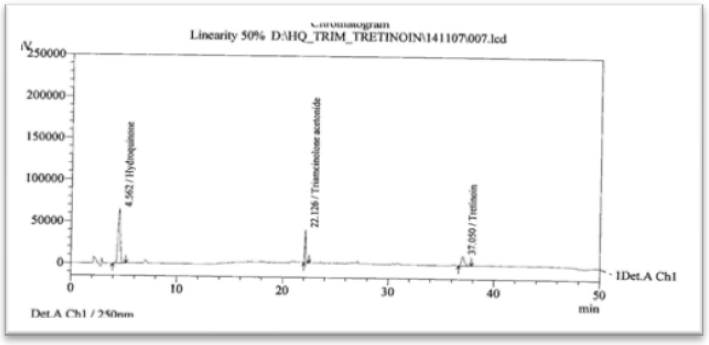


Figure 8: HPLC graph for Linearity 50% standard

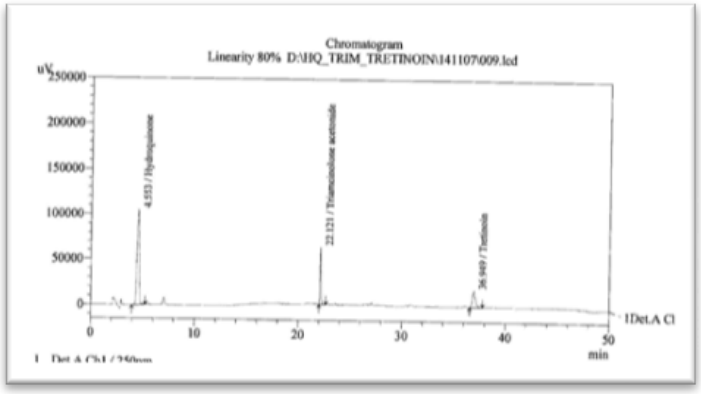


Figure 9: HPLC graph for Linearity 80% standard

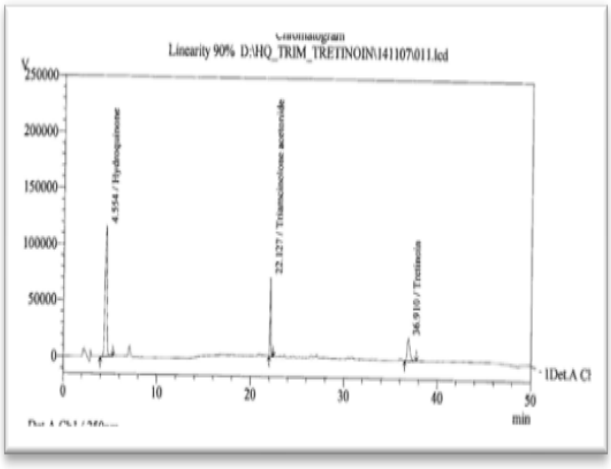


Figure 10: HPLC graph for Linearity 90% standard

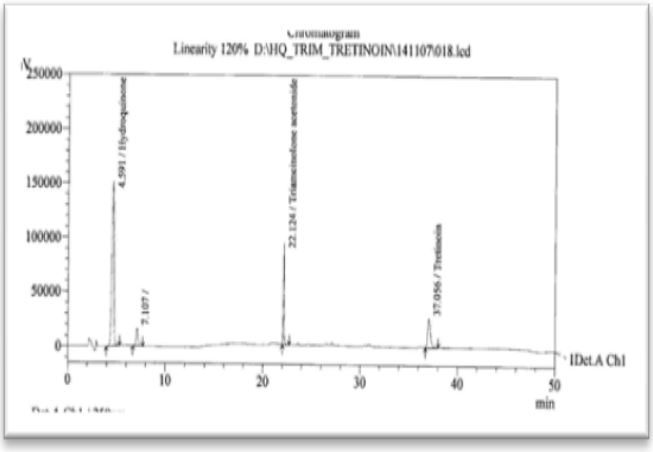


Figure 13: HPLC graph for Linearity 120% standard

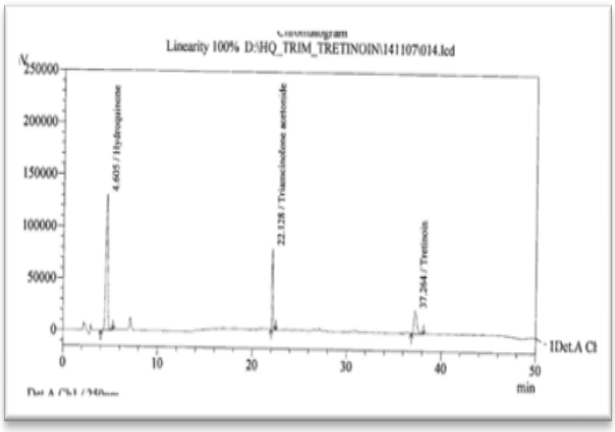


Figure 11: HPLC graph for Linearity 100% standard

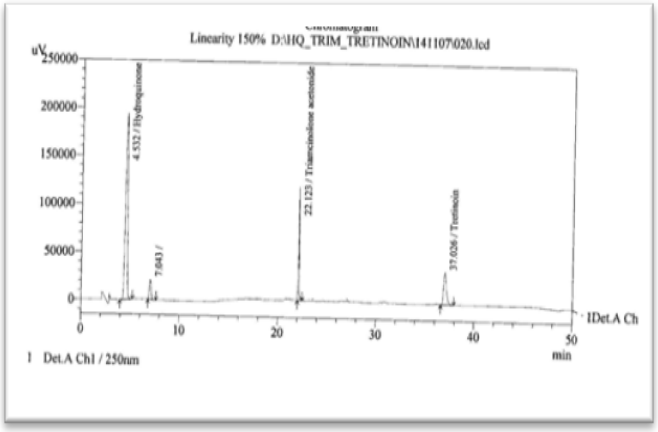


Figure 14: HPLC graph for Linearity 150% standard

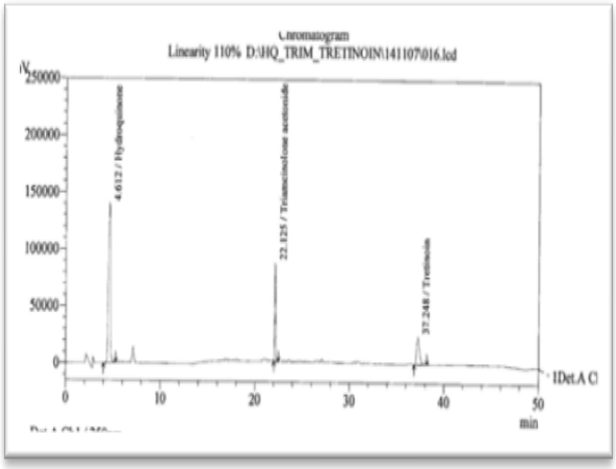


Figure 12: HPLC graph for Linearity 110% standard

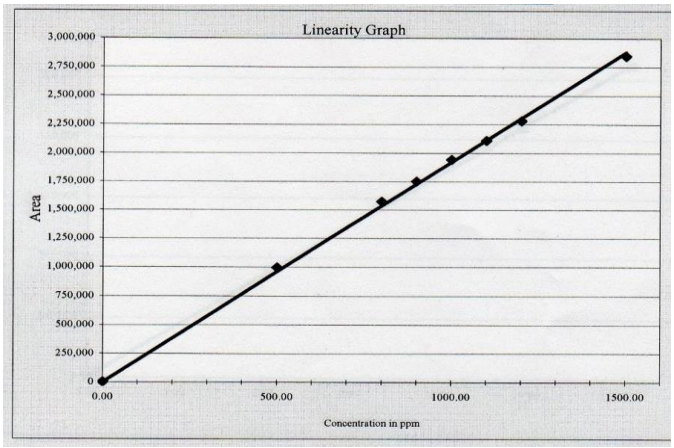


Figure 15: Linearity Graph of Hydroquinone

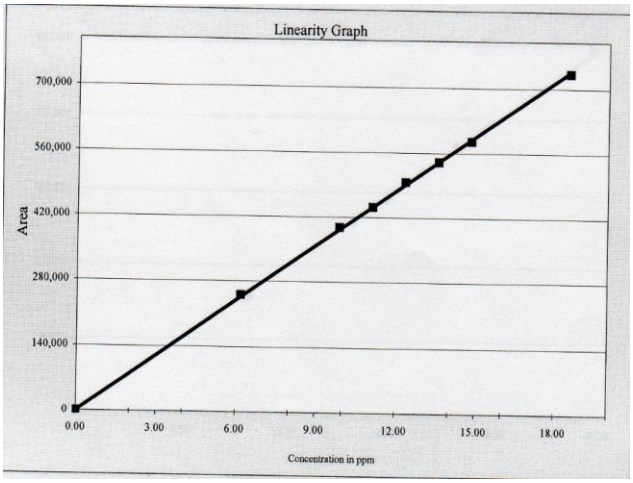


Figure 16: Linearity Graph of Triamcinolone acetonide

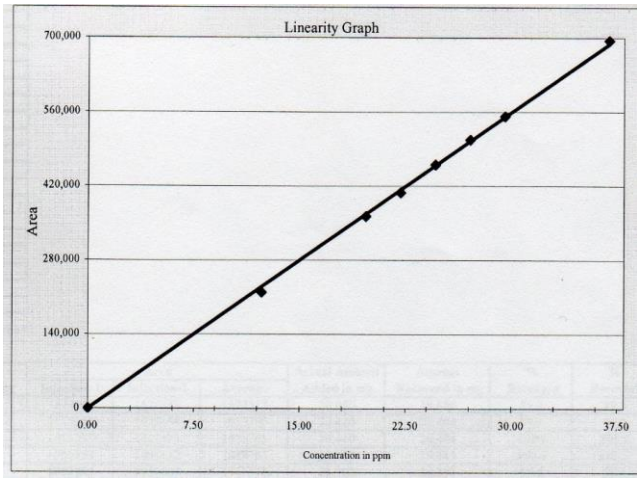


Figure 17: Linearity Graph of Tretinoin

(C) Accuracy:

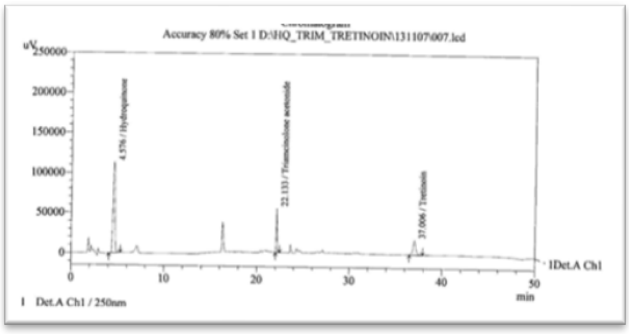


Figure 18: HPLC Chromatogram of accuracy 80%

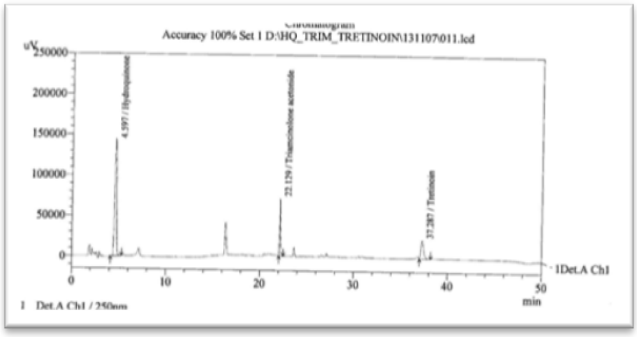


Figure 19: HPLC Chromatogram of accuracy 100%

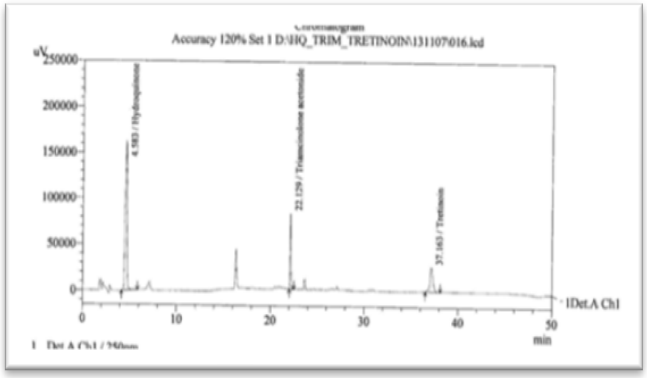


Figure 20: HPLC Chromatogram of accuracy 120%

(D) Specificity:

Preparation of Placebo solution: 1.250gm of placebo cream was accurately weighed and transfers it into a 25ml volumetric flask and 15 ml of methanol was added and sonicated in the ultrasonic bath for 30 minutes with occasional swirling. The volume was made up with methanol. The solution was filtered through 0.45 um Teflon membrane filter.

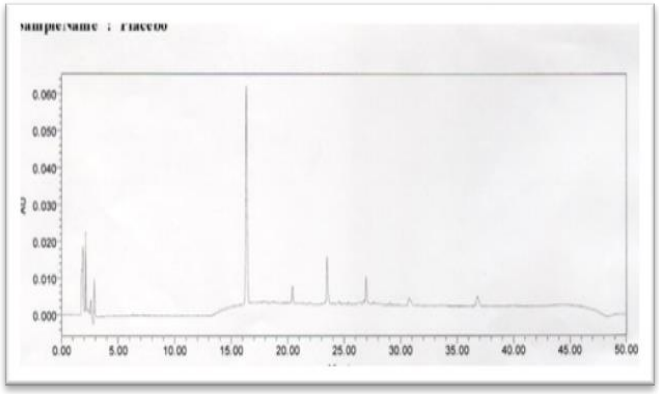


Figure 21: HPLC Chromatogram of Placebo

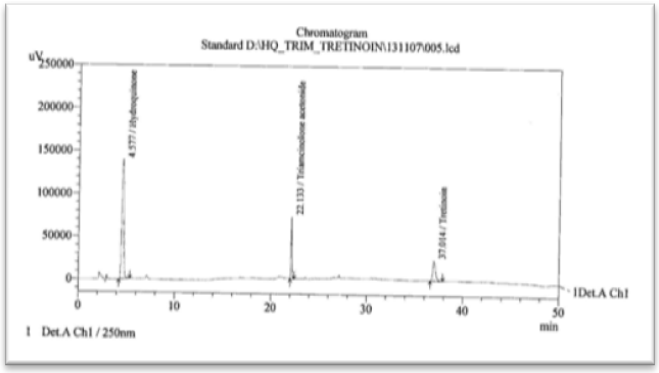


Figure 22: HPLC Chromatogram of Standard

(E) Robustness:

Chromatogram of placebo shows that no interference was found at the retention time of hydroquinone, triamcinolone acetonide and tretinoin.

Table No 6: Robustness of Developed Method

Parameter	Initial Value	Change Valve
Change in pH	4.5	4.3 and 4.7
Change in flow rate	1.0 ml	0.9 ml and 1.1ml
Change in temperature	25 C	20 C and 30 C

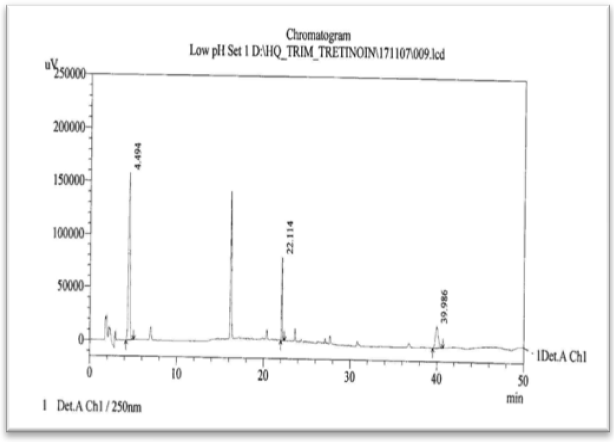
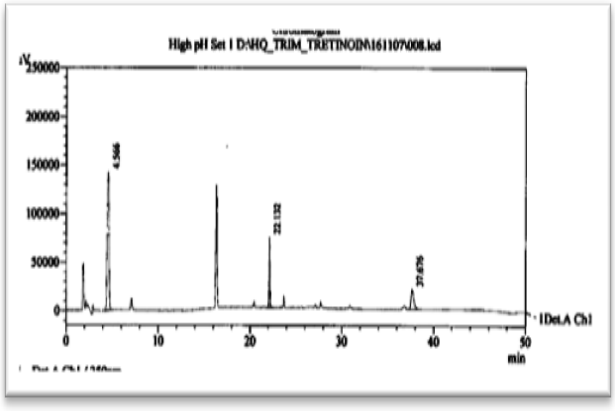


Figure 23: HPLC Chromatogram of Low pH 4.3



. Figure 24: HPLC Chromatogram of High pH 4.7

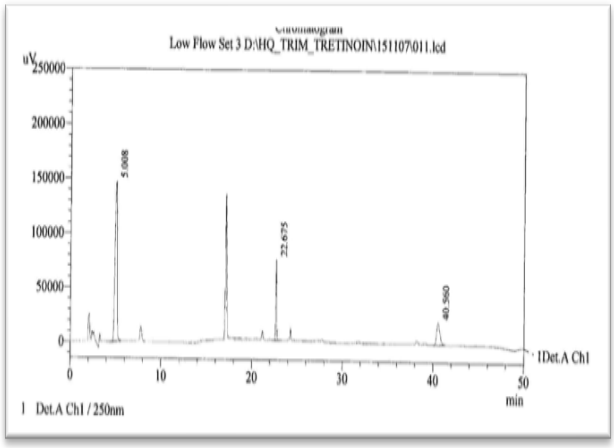


Figure 25: HPLC Chromatogram of Low Flow 0.9 ml/min

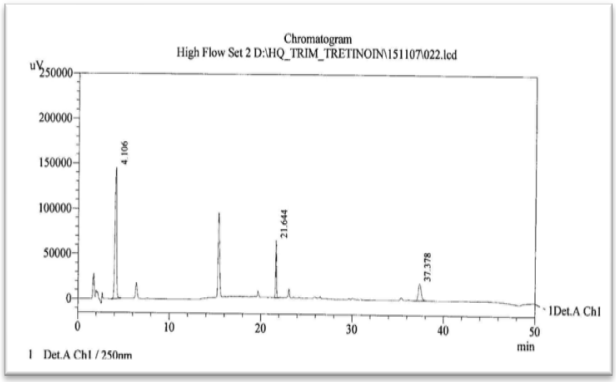


Figure 26: HPLC Chromatogram of High Flow 1.1 ml/min

(F) Ruggedness: By different analysts

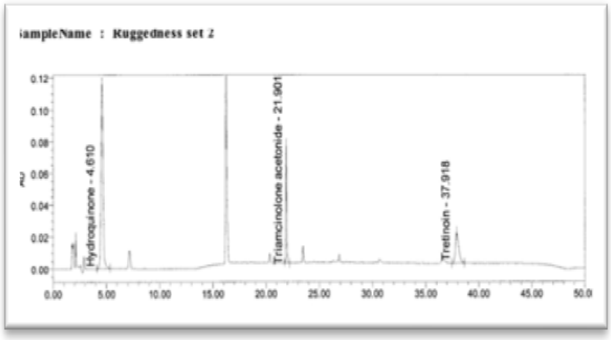


Figure 27: HPLC Chromatogram of Ruggedness

Result and Discussion

On the basis of the experiments, we can conclude that the RP-HPLC developed for the simultaneous determination of hydroquinone, triamcinolone acetonide and tretinoin can be used for routine analysis.

Method Development: Simultaneous determination of hydroquinone, triamcinolone acetonide and tretinoin were estimated by reverse phase HPLC using acetonitrile; tetrahydrofuran and sodium acetate buffer pH 4.5 adjusted with glacial acetic acid and column Lichro CART (250*4.0 mm, 5um) as a stationary phase and the chromatogram of hydroquinone , triamcinolone acetonide and tretinoin has shown in figure and peak was observed at 250 nm which was selected as a wavelength for quantitative estimation.

After development of the method it was validated for specificity, linearity, precision, accuracy Studies. The method was found to be specific because it did not show any interference with placebo solutions. The chromatograms of the specificity are shown in figures. The precision was found to be within the limits. The limit were not more than RSD <2%. Precision RSD was found to be 0.821, 0.254, and 0.851 for hydroquinone, triamcinolone acetonide and tretinoin. This indicates that the method is precise and accurate. The chromatograms for precision are shown in figure7 and the data regarding the precision are showns in tables. From the linearity table it was that the drug obeys beer's law and from the linearity studies, the specified range for hydroquinone was found to be 500ug/ml to 1500ug/ml and for triamcinolone acetonide it was found to be 6.25ug/ml to 18.75ug/ml and for tretinoin it was found to be 12.5ug/ml to 37.5 ug/ml. From the results shown in the accuracy table it was found that recovery value of pure drug from the reanalyzed solution of formulation were between 98.0 % to 102% which indicates that the method is accurate and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods . the system suitability parameter also reveals that values within the specified limit for the proposed method .Theoritical plate for hydroquinone was found to be not less than 2500 , for triamcinolone acetonide it was to be about 250000 and for tretinoin it was found to be not less than 65000.

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Table No : 7 Repeatability of hydroquinone, triamcinolone acetonide and tretinoin

Area			Amount (%)		
Hydroquinone	**Triamcinolone acetonide**	**Tretinoin**	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
1614574	408611	434748	101.6	98.8	99.5
1591397	404721	432159	101	98	99.7
1598490	405261	433713	101.8	98.5	100.5
1592212	409460	437393	99.8	98	99.7
1594741	410433	437162	100.1	98.2	99.7
1619390	407916	442742	101.5	98.3	101.8
AVERAGE			101	98.4	100.2
S.D.			0.829	0.25	0.852
R.S.D.			0.821	0.254	0.851

Table No. 8: Data for linearity level of hydroquinone, triamcinolone acetonide and tretinoin

Linearity Level %	**Average Area**		
	Hydroquinone	**Triamcinolone acetonide**	**Tretinoin**
50%	992886	249751	218955
80%	1570759	397507	362465
90%	1749324	441679	407223
100%	1941752	496001	460103
110%	2105920	539951	506836
120%	2278611	584583	551323
150%	2845382	732243	694116
Slope	1838	38903	19314
Correlation	0.99978	0.99986	0.99992

Table No. 9: Accuracy of hydroquinone, triamcinolone acetonide and tretinoin

Average Area			
Conc.	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
80	1572317	372123	390533
80	1613650	383255	402985
80	1613765	372408	390533
100	1988763	485871	465856
100	1961578	480867	466125
100	1974971	481065	464645
120	2308716	564416	590945
120	2300884	567642	594167
120	2308319	564416	594056

Table No. 10: % recovery of hydroquinone, triamcinolone acetonide and tretinoin

% Recovery			
Conc.	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
80	99.2	98	99.9
80	99.5	100.7	101.3
80	99.9	98.1	99.1
100	100.2	102.3	100.1
100	99.6	101.2	99.7
100	99.7	101.5	98.9
120	98.7	99	101.6
120	98.1	99.6	101.9
120	98.3	99	101.6
Average	99.3	99.9	100.5
S.D	0.768	1.529	1.156
RSD	0.774	1.531	1.15

Table No.11 : Robustness of developed method on low pH (4.3)

Area			Amount (%)		
Hydroquinone	**Triamcinolone acetonide**	**Tretinoin**	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
2043654	440220	447129	101.2	98.0	100.9
2035203	436544	436544	101.5	98.1	99.8
2038456	433566	433566	101.9	97.8	99.0
AVERAGE			101.5	98.0	99.7
S.D.			0.412	0.284	1.052
R.S.D.			0.42	0.291	1.056

Table No. 12: Robustness of Hydroquinone, Triamcinolone acetonide and tretinoin on High pH - 4.7

Area			Amount (%)		
Hydroquinone	**Triamcinolone acetonide**	**Tretinoin**	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
1959291	413906	437814	100.0	98.0	100.9
1971743	418723	440829	99.8	98.2	100.8
1985879	422848	455200	98.6	98.5	102.1
	AVERAGE		99.5	98.3	100.8
	S.D.		1.186	0.510	1.011
	R.S.D.		1.182	0.517	1.023

Table No. 13: Data for robustness of hydroquinone, triamcinolone acetonide and tretinoin on low flow 0.9 ml/min

Area			Amount (%)		
Hydroquinone	**Triamcinolone acetonide**	**Tretinoin**	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
1919825	411315	422546 **Figure 24: HPLC Chromatogram of High pH 4.7**	101.9	98.9	100.0
1869234	404526	410938	100.4	98.3	98.4
1879460	402856	412253	100.6	98.1	98.3
AVERAGE			101.0	98.4	99.1
S. D.			0.776	0.539	1.113
R.S.D.			0.769	0.542	1.102

Table No. 14: Data for robustness of hydroquinone, triamcinolone acetonide and tretinoin on High flow 1.1 ml/min

Area			Amount (%)		
Hydroquinone	**Triamcinolone acetonide**	**Tretinoin**	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
1919825	411315	422546	101.9	98.9	100.0
1869234	404526	410938	100.4	98.3	98.4
1879460	402856	412253	100.6	98.1	98.3
AVERAGE			101.0	98.4	99.1
S. D.			0.776	0.539	1.113
R.S.D.			0.769	0.542	1.102

Table No. 15: Robustness of on low temperature 20^{O}C

Area			Amount (%)		
Hydroquinone	**Triamcinolone acetonide**	**Tretinoin**	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
1607636	411106	410437	101.3	98.4.	100.0
1627701	418460	415601	101.6	98.2	99.6
1592671	409355	409823	101.1	98.7	99.9
	AVERAGE		101.4	98.4	99.8
	S.D.		0.423	0.312	0.711
	R.S.D.		0.427	0.310	0.713

Table No. 16: Robustness on High temperature 30^{O}C

Area			Amount (%)		
Hydroquinone	**Triamcinolone acetonide**	**Tretinoin**	**Hydroquinone**	**Triamcinolone acetonide**	**Tretinoin**
1604352	411234	411156	101.8	98.7	100.1
1595676	4103487	410897	100.5	98.6	99.4
1597654	411298	411554	100.9	98.1	99.6
	AVERAGE		101.1	98.4	99.7
	S.D.		0.676	0.539	0.569
	R.S.D.		0.679	0.542	0.566

Table No. 17: Data for ruggedness of hydroquinone, triamcinolone acetonide and tretinoin

Area			Amount (%)		
Hydroquinone	Triamcinolone acetonide	Tretinoin	Hydroquinone	Triamcinolone acetonide	Tretinoin
1587968	405033	417511	101.3	98.2	98.6
1595216	405038	420656	101.5	98.0	99.1
1600424	405303	420103	102.2	98.3	99.3
1586915	404844	417309	101.2	98.1	98.5
1579900	406320	422894	101.4	98.1	99.5
1589217	406938	425799	101.1	98.4	100.3
AVERAGE			101.4	98.2	100.2
S.D.			0.706	0.248	0.99
R.S.D.			0.698	0.243	0.94

Table No .18: Results with acceptance criteria

S. No.	Parameter	Acceptance criteria	Results obtained		
1	**Specificity**	Should not interfere with placebo	**Passes**		
2	**Linearity**	Correlation coefficient not less than 0.999	**Hydroquinone**	**Trim**	**Tretinoin**
			0.99978	0.99986	0.99992
3	**Precision**	R.S.D.NMT 2	**Hydroquinone**	**Trim**	**Tretinoin**
			0.821	**0.254**	0.851
4	**Accuracy**	R.S.D.NMT 2	**Hydroquinone**	**Trim**	**Tretinoin**
			0.774	**1.531**	**1.150**
		Recovery of the spiked drug (98-102 %)	**Hydroquinone**	**Trim**	**Tretinoin**
			99.3%	**99.9%**	**100.5%**
5	**Ruggedness**	R.S.D.NMT 2	**Hydroquinone**	**Trim**	**Tretinoin**
			0.698	**0.243**	**0.940**
6	**Rubustness** a. Low pH	R.S.D.NMT 2	**Hydroquinone**	**Trim**	**Tretinoin**
			0.420	0.291	1.056
	b. High pH		1.182	0.517	1.023
	c. Low flow		0.527	0.318	0.741
	d. High flow		0.769	0.542	1.102
	e. Low temp.		0.427	0.310	0.713
	f. High temp		0.679	0.542	0.566