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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i39B32185

Editors:
(1) Dr. Rafik Karaman, Al-Quds University, Palestine.

Reviewers:
(1) Shailendra S. Suryawanshi, KLE Academy of Higher Education and Research, India.
(2) Devabrata Saikia, Assam down town University, India.

Complete Peer review History: https://www.sdiarticle4.com/review-history/71807

ABSTRACT

Objective: A simple, sensitive, rapid, precise and accurate stability indicating RP-HPLC method has been developed for simultaneous estimation of Hydroquinone, Hydrocortisone and Tretenoin from their Cream Formulation.

Method: The Chromatographic separation was achieved on a reversed-phase Inertsil C₁₈ (4.6 mm I.D. × 250 mm, 5 µm) column using a mobile phase consisting of Buffer (pH 4.0) 0.05M potassium dihydrogen ortho phosphate-Methanol in the ratio of 80:20% V/V at a flow rate of 1ml/min and UV detection at λmax 265 nm. The method showed linearity with correlation coefficient of Hydroquinone, Hydrocortisone and Tretenoin was 0.998, 0.998 and 0.996 over the range of 40-120 µg/ml, 20-60 µg/ml and 0.25-0.75 µg/ml respectively.

Result: The retention time was 3 min, 5 min and 6 min for Hydroquinone, Hydrocortisone and Tretenoin respectively. The mean recoveries were found to be in the range of 97.00–101.00 % for all the components. The method was validated as per the ICH guidelines. The method was validated as per the ICH guidelines.

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Conclusion: The method was stable and specific and when sample was stressed under different conditions like Acid, Base, Oxidative, Thermal and Photolytic, no interference of degradants were observed.

Keywords: Hydroquinone, Hydrocortisone, Tretenoin, Stability indicating RP-HPLC, Validation.

1. INTRODUCTION

Hydroquinone, Hydrocortisone and Tretenoin combine formulation used in the treatment of Melasma. Melasma is a common pigmentation disorder. In Melasma brown, gray patches or dark spot appear on the skin, especially those exposed to sunlight. Hydroquinone is skin lightening medicine. It reduce the amount of skin pigment that causes darkening of skin [1,2,3].

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 used in hyperpigmentation, melasma, age spot caused by pregnancy.

Hydrocortisone is a steroid medicine (Fig.2). It is used to treat redness, swelling, itching and discomfort of various skin condition [4].

Tretenoin is a vitamin A derivative (Fig.3). Tretenoin is used to treat Acne or skin disease such as wrinkles, dark spot and rough skin [5].

Various analytical methods using UV, HPLC, and stability studies have been mentioned for Hydroquinone alone and in combination with other compounds, according to a review of the literature [6-12]. For Tretenoin alone and with various combinations, analytical methods such as spectrophotometry, HPLC, have been published [6-13].

The literature review revealed that no analytical methods and stability method are reported for simultaneous determination of our interested Cream dosage form. The proposed approach has been validated in accordance with ICH criteria [14-15].

2. MATERIALS AND METHODS

2.1 Instrumentation

HPLC of Cyber Lab (Model: Cyber lab 1600 EX) with Inertsil C₁₈ (4.6 mm I.D. × 250 mm, 5 μm) Column was used for chromatographic separation. It contains Rheodyne 7725i injector and UV Detector (Deuterium). The ultrasonic bath made by Today-Tech was used for sonication. UV Visible spectrophotometer (LT - 2900) made by Labtronics and Analytical balance (BL–220H) made by Shimadzu Ltd. having weighing capacity of 0.01 – 200 gm were used for the study [7].

![Fig.1. Structure of Hydroquinone](image1)

![Fig.2. Structure of Hydrocortisone](image2)

![Fig.3. Structure of Tretenoin](image3)

2.2 Chemicals and Reagents

Pharmaceutically pure samples of Hydroquinone, Hydrocortisone and Tretenoin were obtained as a gift samples from R.K. School of Pharmacy, Loba Chemical Private limited, Mumbai, Abbott Pharmaceutical, Mumbai respectively. Acetonitrile and Methanol were obtained from Merck Specialties Private Limited, Mumbai and Molychem, Mumbai respectively, water was obtained from Loba Chemical Pvt. Ltd., Mumbai and Astron Chemicals, Ahmedabad respectively.
2.3 Chromatographic Condition

HPLC of Cyber Lab (Model: Cyber Lab 1600 EX) with InertsilC18 (4.6 mm I.D. × 250 mm, 5 µm) Column was used for Chromatographic Separation. Standard solutions of Hydroquinone, Hydrocortisone and Tretenoin were injected in column with 20 µL micro-syringe. The chromatogram was run for appropriate minutes with mobile phase Buffer 0.05M potassium dihydrogen ortho phosphate (pH 4.0) Methanol in the ratio of 80:20 %V/V at a flow rate of 1 ml/min and UV detection at $\lambda_{max}$ 265 nm. The chromatogram was stopped after separation achieved completely. Data related to peak like area, height, retention time, resolution etc. were recorded using software. The typical chromatogram of separation of Hydroquinone, Hydrocortisone and Tretenoin was shown in Fig.4 and all chromatographic condition is shown in Table 1.

2.4 Statistical Calculation

Standard deviations and other statistical parameters were performed by use of Microsoft Excel 2007 software.

2.5 Preparation of the Mobile Phase

HPLC grade solvents were used in separate bottle of gradient pumps as mobile phase. A mixture of Buffer and methanol in the ratio of 80:20% v/v were adjusted by gradient pump operated by LC solution software [7]. Mixed solvents were filtered through nylon 0.45µm membrane filter and degassed by the instrument and used as mobile phase.

2.6 Preparation of Buffer

Take about 6.8 gm potassium dihydrogen ortho phosphate reagent into a 1000 ml beaker. Add 800mL water and dissolve. Adjust pH 4.0 of this solution with 1% Orthophosphoric acid. Make up volume up to 1000 ml with water and use this solution as buffer.

2.7 Preparation of Standard Stock Solution

Accurately weighted 40 mg Hydroquinone, 80 mg Hydrocortisone and 5 mg Tretenoin were transferred to 100 ml methanol and diluted up to mark with methanol to prepare standard solution having concentration 400 µg/ml, 800 µg/ml and 5 µg/ml respectively.

2.8 Preparation of Sample Solution

Weight about 4 gm Cream into a 100ml volumetric flask. Add 60 ml methanol and put this volumetric flask on water bath at 60°C for 15 minutes then allow cooling at room temperature. Shake for 15 minutes then make up volume with methanol up to 100mL. Filter this solution with what man filter paper no-1. The solution was diluted up to the mark with methanol.

2.9 Determination of Wavelength of Maximum Absorbance

The standard solution of Hydroquinone 80 µg/ml, Hydrocortisone 40 µg/ ml, Tretenoin 0.50 µg/ ml was scanned in the range of 200 to 400nm and UV Spectrum is recorded.

2.10 Chromatographic Conditions

The chromatographic separations were performed using the final chromatographic conditions as mentioned in Table 1.

3. RESULTS AND DISCUSSION

3.1 HPLC Method Development and Mobile Phase Optimization

Hydroquinone, Hydrocortisone and Tretenoin are combinational cream dosage form used as melasma and anti-acne. This work was focused on the optimization of the conditions for the simple and rapid as well as low cost analysis adding a selection of the proper column and mobile phase. Solvent type, solvent strength, detection wavelength and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile phase conditions were optimized so there was no interference from solvent and excipients.

The aim of this study was to develop a isocratic RP-HPLC assay method and stability study for the analysis of Hydroquinone, hydrocortisone and Tretenoin in cream formulation. Since the drug is soluble in polar solvents like methanol and buffer, a RP-HPLC Method was thought to be suitable. Initially different mobile phases have been tried. The results of the trials are shown in Table 2.
Table 1. Final Chromatographic Conditions

| Sr. No | Parameter          | Description                                    |
|--------|--------------------|------------------------------------------------|
| 1      | Elution            | Isocratic                                      |
| 2      | Mobile Phase       | Buffer 0.05M Potassium Di hydrogen Ortho Phosphate (pH 4.0) Methanol in the ratio of 80:20% V/V |
| 3      | Column             | Inertsil C18 (4.6 mm I.D. × 250 mm, 5 µm)      |
| 4      | Flow Rate          | 1 ml/min                                       |
| 5      | Detection          | UV at 265 nm.                                  |
| 6      | Injection Volume   | 20 µl                                          |
| 7      | Temperature        | Ambient                                        |
| 8      | Retention Time     | Hydroquinone: 3 min   Hydrocortisone: 5 min   Tretenoin: 6 min |
| 9      | Run Time           | 10 min                                         |

Table 2. Results of the initial trials for optimization of mobile phase

| Mobile Phase | Ratio   | Result                                      |
|--------------|---------|---------------------------------------------|
| A mixture of methanol and water | 50:50   | Only one drug retention                      |
| A mixture of ACN and Water       | 50:50   | Only one drug retention                      |
| A mixture of Buffer pH 5 and Methanol | 70:30  | No sharp peak observed                      |
| A mixture of Buffer pH 5 and Methanol | 80:20  | Sharp peak and good resolution              |

Finally, acceptable resolution with reasonable peak shapes and high peak purity was achieved by using a mixture of Buffer 0.05M Potassium Di hydrogen Ortho Phosphate (pH 4.0) Methanol in the ratio of 80:20% V/V with flow rate of 1 ml/min at 265 nm. The method parameters were optimized for the analysis of the formulation. A representative chromatogram is shown in Fig. 3, which satisfies all the system suitability criteria, better resolution of the peak from solvent peak with clear baseline separation.

3.2 Method Validation

Method was validated as per ICH guidelines [13, 14]. The precision was calculated by percentage relative standard deviation. The accuracy was expressed in terms of percent recovery of the known amount of the standards added to the known amount of the pharmaceutical dosage forms. Various validation parameters are performed.

3.2.1 System suitability

It is defined as tests to measure the method that can generate result of acceptable accuracy and precision. The system suitability was carried out after the method development and validation have been completed. The system suitability was assessed by triplicate analyses of the drugs at a concentration of 40, 20, 0.25µg/ml of Hydroquinone, Hydrocortisone and Tretenoin respectively. System suitability parameters were shown in Table 3.

3.2.2 Specificity

Specificity of the method was evaluated by injecting the blank injection, working standard samples into the chromatograph to check the co-elution, if any. At the retention time of 3.8, 5.8 and 6.6 min, the proposed method was specific for the detection of Hydroquinone, Hydrocortisone, Tretenoin respectively. There were no peaks at the retention time of Hydroquinone, Hydrocortisone, Tretenoin.

3.2.3 Linearity

Linearity was tested in the concentration range 40, 60, 80, 100, 120 µg/ml for Hydroquinone; 20, 30, 40, 50, 60 µg/ml for Hydrocortisone and 0.25, 0.37, 0.50, 0.62, 0.75 µg/ml for Tretenoin. All the solutions were chromatographed six times in accordance with the ICH. Separates calibration plots for Hydroquinone, Hydrocortisone and Tretenoin were constructed by the plotting peak area against respective concentration and the method was evaluated by determination of the correlation coefficient and intercept, calculated in the corresponding statistical study, correlation coefficient $r^2$ values > 0.999 and intercept very close to zero confirmed the good linearity of the method. The represented data was shown in below Fig. 5, 6, 7 and Table 4.
Table 3. System Suitability data for drug

| Retention Time | Area    | % Area | Height | Asymmetry | Efficiency | Resolution |
|----------------|---------|--------|--------|-----------|------------|------------|
| 3.833          | 2208.106| 45.792 | 265.51 | 1.290     | 4359       | -          |
| 5.837          | 2268.338| 47.041 | 224.64 | 1.417     | 7372       | 7.947      |
| 6.647          | 345.636 | 7.168  | 27.64  | 1.391     | 6548       | 2.698      |

Fig. 4. Typical Chromatogram of blank

Fig. 5. Typical chromatogram of standard

Fig. 6. Typical chromatogram of sample
Table 4. Linearity data for drug

| Hydroquinone | Hydrocortisone | Tretenoin |
|--------------|---------------|-----------|
| Conc. (µg/ml) | Area          | Conc. (µg/ml) | Area          | Conc. (µg/ml) | Area |
| 40           | 1210.56       | 20         | 1162.40      | 0.25         | 167.45 |
| 60           | 1737.94       | 30         | 1667.90      | 0.37         | 247.78 |
| 80           | 2407.03       | 40         | 2309.41      | 0.50         | 343.62 |
| 100          | 2946.17       | 50         | 2828.38      | 0.62         | 420.90 |
| 120          | 3547.34       | 60         | 3404.34      | 0.75         | 489.50 |

Fig. 7. Linearity plot of hydroquinone

Fig.8. Linearity plot of hydrocortisone

Fig.9. Linearity plot of Tretenoin
3.2.4 Precision

It is a measure of degree of repeatability of an analytical method under normal operation and it is normally expressed as RSD. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits Intermediate precision [7]:

Precision was performed on different day by using same column. The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. The %RSD for the area of five replicate injections was found to be within the specified limits [7]. Result was shown in Table 5.

3.2.5 Accuracy

To check the accuracy of proposed method, recovery studies were carried out from pre analyzed samples at three different levels of standard addition 80%, 100% and 120% of label claim. The validity and reliability of proposed method was assessed by recovery studies by standard addition method. Results of Accuracy were presented in the Table 6,7,8.

3.2.6 Robustness

Robustness of the method was determined by making slight changes in the chromatographic conditions. It was observed that there were no marked changes in the chromatograms, which demonstrated that the RP-HPLC method developed, are rugged and robust. The result of robustness is given in Table 9.

3.2.7 LOD and LOQ

LOD and LOQ were calculated from the formula 3.3 x (σ/S) and 10 x (σ/S), respectively where, σ is standard deviation of intercept and S is the mean of slope. LOD and LOQ condition are shown in Table 10.

3.2.8 Assay of the Pharmaceutical Preparation

The suggested methods were successfully applied for the determination of Hydroquinone, Hydrocortisone and Tretenoin in its pharmaceutical cream formulation. The results, shown in Table 11 were satisfactory and agreed with the labeled amounts. Since no analytical method (reference) is available for the assay of this triple mixture.

Table 5. Precision study for Hydroquinone, Hydrocortisone and Tretenoin

| Drug       | % Mean Label Claim | Standard Deviation | % Relative Standard Deviation | Standard Error |
|------------|--------------------|--------------------|-------------------------------|---------------|
| Repeatability Data |                    |                    |                               |               |
| Hydroquinone | 99.16              | 0.68               | 0.69                          | 0.28          |
| Hydrocortisone | 99.50              | 0.50               | 0.50                          | 0.20          |
| Tretenoin | 99.33              | 0.75               | 0.75                          | 0.30          |
| Intra-day Precision |                    |                    |                               |               |
| Hydroquinone | 99.91              | 0.44               | 0.44                          | 0.18          |
| Hydrocortisone | 99.94              | 0.86               | 0.86                          | 0.35          |
| Tretenoin | 100.09             | 0.66               | 0.66                          | 0.27          |
| Inter-day Precision |                    |                    |                               |               |
| Hydroquinone | 100.09             | 0.56               | 0.55                          | 0.22          |
| Hydrocortisone | 10.10              | 0.88               | 0.88                          | 0.36          |
| Tretenoin | 99.8               | 0.68               | 0.68                          | 0.28          |

Table 6. Accuracy data for Hydroquinone

| Concentration | Amount present in sample (eq. wt) (µg/ml) | Amount of std added (µg/ml) | Total Amt of present (µg/ml) | Total Amount found (µg/ml) | Recovered Amount | % Recovery |
|---------------|------------------------------------------|-----------------------------|-----------------------------|----------------------------|------------------|------------|
| 80            | 80                                       | 64                          | 144                         | 142                        | 63.5             | 99.21      |
| 100           | 80                                       | 80                          | 160                         | 156                        | 79               | 98.75      |
| 120           | 80                                       | 96                          | 176                         | 175                        | 95.5             | 99.47      |
Table 7. Accuracy data for Hydrocortisone

| Concentration Level | Amount Present in Sample (eq. wt µg/ml) | Amount of Std Added (µg/ml) | Total Amount of Present (µg/ml) | Total Amount Found (µg/ml) | Recovered Amount | % Recovery |
|---------------------|---------------------------------------|----------------------------|--------------------------------|---------------------------|------------------|------------|
| 80                  | 40                                    | 32                         | 72                             | 71.5                      | 31.5             | 98.43      |
| 100                 | 40                                    | 40                         | 80                             | 79.5                      | 39.9             | 99.75      |
| 120                 | 40                                    | 48                         | 88                             | 87.6                      | 47.5             | 98.95      |

Table 8. Accuracy data for Tretenoin

| Concentration Level | Amount Present in Sample (eq. wt µg/ml) | Amount of Std Added (µg/ml) | Total Amount of Present (µg/ml) | Total Amount Found (µg/ml) | Recovered Amount | % Recovery |
|---------------------|---------------------------------------|----------------------------|--------------------------------|---------------------------|------------------|------------|
| 80                  | 0.50                                  | 0.40                       | 0.9                            | 0.8                       | 0.39             | 97.5       |
| 100                 | 0.50                                  | 0.50                       | 1                              | 0.9                       | 0.49             | 98         |
| 120                 | 0.50                                  | 0.60                       | 1.1                            | 1.0                       | 0.59             | 98.33      |

Table 9. Robustness data for Hydroquinone, Hydrocortisone, Tretenoin

### Change in Flow Rate

| Flow Rate | Hydroquinone | Hydrocortisone | Tretenoin |
|-----------|--------------|----------------|-----------|
| 1 ml/min  | +0.2         | 5.5            | 3.6       | 96.49     | 3.8          | 100        | 6.3       | 99.45     |
| 1 ml/min  | 0            | 5.7            | 3.8       | 100       | 4.0          | 105        | 6.9       | 104.5     |
| 1 ml/min  | -0.2         | 6.1            | 4.0       | 107.01    | 105          | 6.9        | 104.5     |

### Change in Mobile phase

| Buffer (pH 4) | Methanol 80:20 | Change | Hydroquinone | Hydrocortisone | Tretenoin |
|---------------|-----------------|--------|--------------|----------------|-----------|
| Buffer (pH 4)| +2%             | 5.4    | 3.6          | 94.73          | 6.2       | 94.73     |
| Buffer (pH 4)| 0               | 5.7    | 3.8          | 100            | 6.6       | 100       |
| Buffer (pH 4)| -2%             | 6.0    | 4.0          | 105.2          | 6.9       | 104.5     |

### Change in pH

| pH 4         | Hydroquinone | Hydrocortisone | Tretenoin |
|--------------|--------------|----------------|-----------|
| +0.2         | 5.7          | 3.8            | 100       | 6.6       | 100       |
| 0            | 5.7          | 3.8            | 100       | 6.6       | 100       |
| -0.2         | 5.8          | 3.8            | 100       | 6.6       | 100       |

Table 10. LOD and LOQ data for hydroquinone, hydrocortisone and Tretenoin

| Component     | LOD (µg/ml) | LOQ (µg/ml) |
|---------------|-------------|-------------|
| Hydroquinone  | 0.07        | 0.2         |
| Hydrocortisone| 0.04        | 0.1         |
| Tretenoin     | 0.001       | 0.005       |

Table 11. Assay of the pharmaceutical cream formulation

| Brand/Drug   | % Mean Recovery | Standard Deviation | % Relative Standard Deviation | Standard Error |
|--------------|-----------------|--------------------|------------------------------|---------------|
| Aret-hc      | Hydroquinone    | 98.12              | 0.31                         | 0.31          | 0.21         |
| Aret-hc      | Hydrocortisone  | 97.08              | 0.62                         | 0.64          | 0.44         |
| Cream        | Hydrocortisone  | 98.33              | 0.2                          | 0.20          | 0.14         |
| Cream        | Tretenoin       | 98.33              | 0.2                          | 0.20          | 0.14         |
Table 12. Summary of validation parameters

| Sr No | Parameter          | Hydroquinone    | Hydrocortisone | Tretenoin   |
|-------|--------------------|-----------------|----------------|-------------|
| 1     | Limit of Linearity | 40-120 µg/ml    | 20-60 µg/ml    | 0.25-0.75 µg/ml |
| 2     | Regression Equation| Y=11.33x+5.9586 | Y=2362x+6.107  | Y=330.8x+2.488 |
|       | Slope              | 11.33           | 2362           | 330.8       |
|       | Intercept          | 5.958           | 6.107          | 2.488       |
|       | Correlation Coefficient | 0.999    | 0.999          | 0.998       |
| 3     | Accuracy           | 98-99 %         | 98-99 %        | 97-98 %     |
| 4     | Precision          | 0.69            | 0.50           | 0.75        |
|       | Intraday           | 0.44            | 0.86           | 0.66        |
|       | Interday           | 0.55            | 0.88           | 0.68        |
| 5     | LOD(µg/ml)         | 0.07            | 0.04           | 0.001       |
| 6     | LOQ(µg/ml)         | 0.2             | 0.1            | 0.005       |
| 7     | Assay              | 98.12           | 97.08          | 98.33       |

3.2.9 Force Degradation Studies

Degradation studies as per ICH Guideline [13, 14]. The drug was intentionally degraded by treating with Acid, Base, Thermal, Oxidation and exposing to Sunlight condition. Degradation condition is shown in Table 13.

3.2.9.1 Acid degradation

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

3.2.9.3 Oxidation degradation

For Oxidative Degradation blank preparation 2 ml of 3% H\textsubscript{2}O\textsubscript{2} was taken and volume makes up to 10 ml with mobile Phase. For hydroquinone, hydrocortisone and Tretenoin, 1 ml stock solution was taken and 3 % H\textsubscript{2}O\textsubscript{2} was added and kept for 3 hrs. Finally volume was made up to 10ml with mobile phase. For sample degradation also same procedure was followed.

3.2.9.4 Thermal degradation

For Thermal Degradation blank preparation, 2 ml mobile phase was kept at 105°C and then volume make up to 10 ml with mobile phase. For hydroquinone, hydrocortisone and Tretenoin, 1 ml stock solution was taken and kept at 105°C for 4.5 hours. Finally volume was made up to 10ml with mobile phase. For sample degradation also same procedure was followed.

Table 13. Result of degradation study

| Condition | Hydroquinone | Hydrocortisone | Tretenoin |
|-----------|--------------|----------------|-----------|
| Acid      | 30.36        | 34.03          | 17.84     |
| Base      | 45.01        | 22.54          | 21.48     |
| Oxidation | 28.96        | 25.25          | 31.72     |
| Photo     | 36.25        | 25.25          | 25.82     |
| Thermal   | 35.99        | 26.63          | 28.27     |
3.2.9.5 Sunlight degradation

For Sunlight Degradation, 1 ml Hydroquinone, Hydrocortisone and Tretenoin were taken and kept at sunlight for 3.5 hours. Finally volume was made up to 10ml with mobile phase. For sample degradation also same procedure was followed.

4. CONCLUSION

The Results of our study indicate that the proposed RP-HPLC Method is simple, rapid, precise and accurate. This Method was developed and validated for the routine analysis of Hydroquinone, Hydrocortisone and Tretenoin in cream topical formulation. The result reveals that the proposed method could be successfully applied for the routine analysis and quality control of pharmaceutical dosage forms containing Hydroquinone, Hydrocortisone and Tretenoin. The mobile phase conditions were optimized so there was no interference from solvents and excipients. The mobile phase contain Buffer 0.05M potassium dihydrogen orthophosphate (pH 4.0)-Methanol in the ratio of 80:20% V/V at a flow rate of 1 ml/min was selected. To determine the appropriate wavelength for simultaneous estimation of Hydroquinone, Hydrocortisone and Tretenoin solution of these compounds in methanol were scanned in the range of 200 – 400 nm. From the overlay UV spectra it concluded that 265 nm was the most appropriate wavelength for analysis of all the drugs with suitable sensitivity.
Statistical analysis proves that the method is repeatable and selective for the analysis of this cream formulation. It can therefore be concluded that use of the method can save much time and money and it can be used in small laboratories with very high accuracy and a wide linear range. The method was found to be stable, as there was less degradation observed when the drug were stressed under accelerated conditions.

**DISCLAIMER**

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

**CONSENT**

It is not applicable.

**ETHICAL APPROVAL**

It is not applicable

**ACKNOWLEDGEMENTS**

We would like to express our gratitude to R.K. School of Pharmacy, Loba Chemical Private limited, Mumbai, Abbott Pharmaceutical, Mumbai for providing the gift samples. We would also like to acknowledge School of Pharmacy, RK University, Rajkot and Department of pharmaceutical sciences, Saurashtra University, Rajkot for providing the necessary infrastructure required to carry out the present research work.

**COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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