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# Validated stability indicating RP-HPLC method for the simultaneous determination of ofloxacin, ornidazole, clobetasol propionate, terbinafine hydrochloride, methyl paraben, propyl paraben in bulk and pharmaceutical dosage form

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## ABSTRACT

A new simple, precise, accurate and selective RP-HPLC method has been developed and validated for simultaneous estimation of Ofloxacin (OFL) ,Ornidazole (ORD), Terbinafine hydrochloride (TFH) , clobetasol propionate(CLP) , Methyl paraben(MP) ,propyl paraben(PP) in bulk and pharmaceutical dosage forms. The method was carried out on a Zodiac $C_{18}$ (250mm x 4.6mm, 5µm) column with a mobile phase consisting of Ortho phosphoric acid buffer, $P^H$ 2.5 and Acetonitrile in the ratio (82:18v/v) and flow rate of 1ml/min .The detection was carried out at 255nm. The retention time for estimation of ofloxacin (0.712min), ornidazole(1.933min), Terbinafine hydrochloride (7.302min), clobetasol propionate (9.224min), Methyl paraben (4.074min), propyl paraben(7.926min) .The Linearity of proposed method was investigated in the range of 1-960 µg/ml with $r^2$ value for ofloxacin (0.999), ornidazole(0.999), Terbinafine hydrochloride (0.999), clobetasol propionate (0.998), Methyl paraben (0.998), propyl paraben(0.997) .The amount of drug estimated by the proposed method was found to be in good agreement with label claim. The developed method was validated for precision, accuracy, sensitivity, robustness and ruggedness. Hence it can be applied for routine analysis of titled drug in bulk and pharmaceutical formulations.

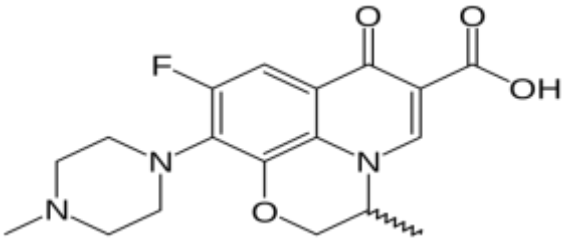
**Keywords**: ofloxacin, ornidazole, Terbinafine Hcl, Clobetasol propionate, methyl paraben, propyl paraben, RP-HPLC, Validation

## INTRODUCTION

### Ofloxacin

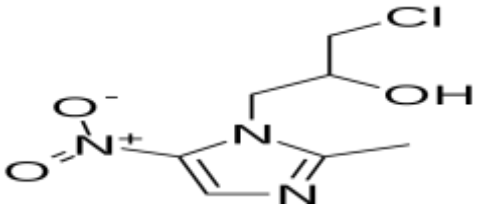
Ofloxacin is a fluoro quinolone derivative. Chemically, it is (RS)-7-fluoro-2-methyl-6-(4-methylpiperazin-1-yl)-10-oxo-4-oxa-1-azatricyclotrideca-5(13),6,8,11-tetraene-11-carboxylic acid and it is used in the treatment of urinary tract, prostate, skin, and respiratory tract infections [1]. Ofloxacin is also used as an antibacterial agent in the treatment of infections caused by a wide range of both Gram-positive and Gram-negative bacteria as well as Chlamydia infections [2].It is soluble in glacial acetic acid ,1,2 di-chloromethane, chloroform ,carbon tetra chloride,

slightly soluble in methanol and water [3]. The drug is official in Indian pharmacopoeia and approved in 1990 on December 28 by U.S- FDA [1].



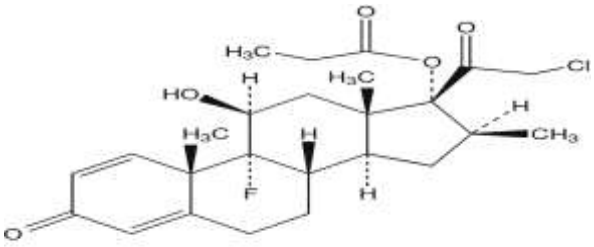
## Ornidazole

Ornidazole is chemically 1-chloro-3-(2-methyl-5-nitro-1H-imidazol-1-yl) propan-2-ol and it is a drug that cures some protozoan infections [4]. It has been investigated for use in Crohn's disease after bowel resection [5]. It is soluble in chloroform and methanol [6].



## Clobetasol propionate

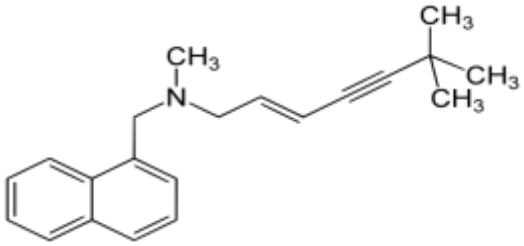
Clobetasol propionate (21-Chloro-9-fluoro-11β-hydroxy-16β-methyl-3, 20-dioxopregna-1, 4-dien-17-yl propanoate) is derivative of prednisolone with high glucocorticoid activity and low mineralocorticoid activity. It is reported in pharmacopoeias such as BP and USP [7, 8]. It is freely soluble in methylene chloride, soluble in methanol, sparingly in alcohol, very sparingly in water [9].



clobetasol propionate

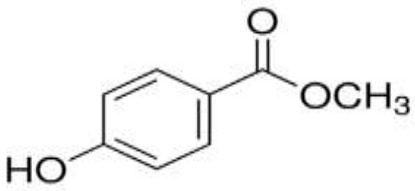
## Terbinafine hydrochloride

Terbinafine hydrochloride (TFH) is a synthetic allylamine antifungal. chemically it is [(2E)-6, 6-dimethylhept-2-en-4-yn-1-yl] (methyl) (naphthalen-1-ylmethyl) amine [10]. It is freely soluble in methanol and dichloromethane, soluble in ethanol, and slightly soluble in water and it is used in the treatment of skin diseases [10]. The drug is official in British pharmacopoeia and approved in 1996 by U.S-FDA [10]



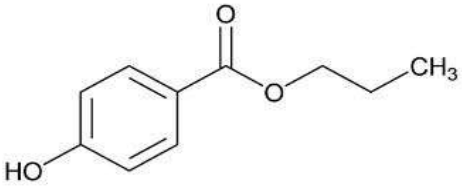
### Methyl Paraben

Parabens are commonly used as preservatives in pharmaceutical, because of their anti-fungal and anti-bacterial properties. It is methyl ester of p-hydroxy benzoic acid; IUPAC Name is Methyl 4-hydroxybenzoate. Methyl paraben is an anti-fungal agent often used in a variety of cosmetics and pharmaceutical products [11]. Freely soluble in water, sparingly soluble in ethanol (95%) [12].




### Propyl paraben

Parabens are commonly used as preservatives in pharmaceutical. Propyl paraben is an n-propyl ester of *p*-hydroxybenzoic acid; IUPAC name is propyl 4-hydroxybenzoate [13]. It is soluble in Acetone, Ethanol, Ether**,** Propylene Glycol and insoluble in Water [14].




In Literature survey revealed that RP-HPLC methods were reported for Ofloxacin and Ornidazole [15]. RP-HPLC method for determination of clobetasol propionate [16] and Terbinafine Hcl [17] alone. Assay [18] and Evaluation methods [19] by using HPLC were reported for determination of Methyl paraben and Propyl paraben in pharmaceutical and cosmetic products. However, no reports are been found in the literature for the simultaneous determination of Ofloxacin, Ornidazole, Terbinafine Hcl , Clobetasol propionate, Methyl paraben ,Propyl paraben in Pharmaceutical preparations.

## MATERIALS AND METHODS

### Chemicals

Ofloxacin ,Ornidazole, Terbinafine Hcl ,Clobetasol propionate was gifted by Darwin Formulations Pvt Ltd ,Vijayawada , AP. Methyl paraben ,Propyl paraben and HPLC grade water from Cystron Pharmaceuticals. Methanol (HPLC grade), Acetonitrile (HPLC grade), Ortho Phosphoric Acid (HPLC grade) were purchased from Merck (Mumbai).

### Instrumentation

Analysis was performed on Waters HPLC 717 plus with UV Detector and Waters HPLC 996 with PDA Detector, equipped with auto sampler and Zodiac $\mathbf{C_{18}}$ column compartment with Empower 2 software. Other equipment used in the study was Analytical Balance (DENVER) and Ultra Sonic bath.

### Chromatographic Conditions

Zodiac $C_{18}$ column (50mm x 4.6mm, 5µm) was used for chromatographic separation.The mobile phase composed of Ortho phosphoric acid buffer and Acetonitrile in the ratio (82:18v/v); at a flow rate of 1ml/min with run time 15mins.The detection of drugs was carried out at 255nm.

### Method development

### Preparation of Buffer:

1ml Ortho Phosphoric Acid in 1 litre water, Filtered and degas.

**Preparation of Diluent:** Methanol was used as a diluent.

### Preparation of standard Solutions

30 mg of ofloxacin, 80 mg of ornidazole, 16 mg of

clobetasol propionate, 30 mg of Terbinafine Hcl, 80 mg of methyl paraben, 8mg of propyl paraben working standards was weighed accurately in a100ml volumetric flask. To this 50ml of methanol was added and sonicated to dissolve, and then it was made up to 100ml. Further 5ml of the above obtained solution was taken and diluted to 50ml with Methanol .Standard was prepared by adding 5ml of each 6 solutions to 50 ml volumetric glass and diluted up to the mark.

Standard stock solutions of 30 mg of OFL , 80 mg of ORD, 16 mg of CLP, 30 mg of TFH , 80 mg of MP and 8mg of PP were prepared using Methanol as diluent . The stock solutions were diluted with diluent (Methanol) to give working standard solutions containing OFL(8-96 µg/ml), ORD(80-960 µg/ml), CLP(1-20 µg/ml), TFH(29-348 µg/ml), MP(8-96 µg/ml), PP(1-9.72 µg/ml) concentrations. These solutions were filled into vials and placed in vial holder. The linearity was determined separately for OFL, ORD, CLP, TFH, MP, and PP by injecting eight concentrations of drug prepared in diluent and calibration curve was constructed by plotting area against the respective concentrations.

## VALIDATION METHOD

The HPLC method was validated in accordance with ICH guidelines. The system precision of the method was verified by six replicate injections of standard solution containing OFL, ORD, CLP, TFH, MP, and PP. The method precision was carried out for the analyte six times using the proposed method. Repeatability was measured by multiple injections of homogenous sample of OFL, ORD, CLP, TFH, MP, and PP. Accuracy was carried out by percentage recovery studies at three different concentration levels. To the pre-analyzed sample solution of OFL, ORD, CLP, TFH, MP, PP and a known amount of standard drug powders of OFL, ORD, CLP, TFH, MP, and PP were added at 50, 100, 150% level. Sensitivity of the proposed method was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) and was determined using the formulae; LOD =3.3 × ASD/S and LOQ = 10 × ASD/S, where, ASD is the average standard deviation and S is the slope of the line.

The robustness of the method was studied for the sample. Ruggedness of the method was performed by two different analysts using same experimental and environmental conditions. It was performed by 50µg/ml. The system suitability parameters such as number of theoretical plates and tailing factor were studied.

Stability of sample solution was established by the storage of sample solution at $25^0$c for 24hr and sample was reanalyzed after 24 hr. Sample solution was reanalysed after 12 hrs and 24 hrs time intervals and assay was determined for these Drug samples.

### Analysis of formulation

1000mg of cream (TERBIFORCE$^{TM}$-PLUS) was weighed accurately in a 25ml volumetric flask. To this 15ml of methanol was added and sonicated to dissolve, and then make up to the mark. The obtained solutions were filtered through 0.45µ Nylon syringe filter. Further the 5ml was transferred into a 50ml calibrated flask and diluted to volume with Methanol and volume was made up to the mark with diluent to obtain a concentration of OFL (8 µg/ml), ORD (8 µg/ml ), CLP (1 µg/ml ), TFH (29 µg/ml ), MP (8 µg/ml), PP (1 µg/ml ) which was then subjected to proposed method and the amounts of OFL, ORD, CLP, TFH, MP, and PP were determined using calibration curves.

### Forced Degradation Studies

The specificity of the method was demonstrated through forced degradation studies conducted on the sample using acid, alkali, oxidative, reductive, thermal, photolytic, Heat, Humidity, Hydrolysis in order to evaluate the ability of the proposed method to separate OFL, ORD, CLP, TFH, MP, PP from both known and unknown degradation product.

Acid degradation was conducted using 1g of sample in a 25ml volumetric flask, 2mL of 5N hydrochloric acid, and alkali degradation was carried out in 2mL of 5N sodium hydroxide, 20ml of diluent was added. The stressed solutions were kept in water bath for 10min and cooled to room temperature (RT), neutralized and then diluted by diluent.

Oxidation degradation was performed by adding 3mL of 30% $H_2O_2$.

For reduction 5ml of 10% sodium bicarbonate was added and kept in water bath for 30min, and cooled to Room Temperature, then diluted by using diluent.

For photolytic degradation sample solution was kept in sunlight for 1hr and then diluted by diluent.

For humidity 5g of sample solutions were kept at 105ºC in oven for 1hr, and diluted by using diluent.

For heat degradation study sample solutions were heated on mantle at 60ºC for 30 mins and diluted by using diluent.

Hydrolysis degradation study to sample solutions 5ml of water and 30ml of diluent and kept on water bath for half an hour and diluted by using diluent.

For thermal studies, 30 ml of diluent was heated on water bath for 60 min and diluted by using diluent.

## RESULTS

The proposed chromatographic system was found suitable for effective separation and quantitation of OFL (0.712min), ORD (1.933min), TFH (7.302min), CLP (9.224min), MP (4.074min), and PP (7.926min) with good resolution, peak shapes and minimal tailing. The overlay UV spectra and typical chromatogram was shown in figure 1 and 2.

The individual chromatograms for OFL, ORD, CLP, TFH, MP and PP were shown in Figure 3, 4, 5, 6, 7, 8. The drugs was found to give linear detector response in the concentration range under study with correlation coefficient of 0.997 -0.999.The samples had followed linearity in the concentration range of OFL(8-96 µg/ml), ORD(80-960 µg/ml), CLP(1-20 µg/ml), TFH(29-348 µg/ml), MP(8-96 µg/ml), PP(1-9.72 µg/ml) were shown in Figure 9, 10,11, 12, 13,14. Percent recoveries for OFL (99.9-101.3%), ORD (99.9-100.7%), CLP (100-101.4%), TFH (99.8-100.7%), MP (99-101.8%), PP (100-100.9%).

The method precision and inter-day precision were evaluated on the basis of % RSD value and found to be in the range 0.316-1.08. As the RSD values were < 2%, the developed method was found to be precise(Table 1).The accuracy of the method studied at three different concentration levels i.e. 50, 100, 150% showed acceptable recoveries in the range of 99-101.8% (Table 2).The LOD for OFL (1.83 µg/mL), ORD (9.52 µg/mL), CLP (0.566 µg/mL), TFH (4.37 µg/mL), MP (1.66 µg/mL) and PP (0.123 µg/mL). Further the LOQ for OFL (5.57) µg/mL, ORD (28.8) µg/mL, CLP (1.717) µg/mL, TFH (13.26) µg/mL, MP (5.035) µg/mL and PP (0.374) µg/mL respectively.

Robustness of the method was studied by making deliberate changes in the chromatographic conditions like flow rate (± 0.2 mL/min), wave length (± 5nm) and mobile phase composition (± 5%). The validation parameters were summarized in (Table 3).

The results of robustness study of the developed method was validated by change in flow rate ,change in wave length and change in mobile phase ratio and the % RSD of those variations are less than 2 (Table 4). When the method was performed by two different analysts under the same experimental and environmental conditions it was found to be rugged and % RSD (<2%) indicating ruggedness of the method.

The system suitability parameters such as number of theoretical plates and tailing factor were studied. Stability of sample solution was established by the storage of sample solution at $25^0$c for 24hr and sample was reanalyzed after 24 hr and assay was determined. The results were shown in (Table 6), Results of Forced degradation studies were shown in (Table 7).

Six replicates of sample solutions containing of OFL (8 µg/mL), ORD (8 µg/mL), CLP (1 µg/mL), TFH (29 µg/mL), MP (8 µg/mL), and PP (1 µg/mL) were injected for quantitative analysis. The amounts of OFL, ORD, CLP, TFH, MP, and PP were found to be 101.3, 99.5, 102, 98, 100.5 and 101% respectively. A good separation and resolution of both drugs indicates that there was no interference from the excipients commonly present in pharmaceutical combined dosage formulations. The results were shown in (Table 5).

## DISCUSSION

The developed method was found suitable for simultaneous estimation of OFL, ORD, CLP, TFH, MP and PP with good peak shapes and minimal tailing. The peak area of the drugs was reproducible as indicated by low coefficient of variance indicating the repeatability of the proposed method. High correlation coefficient of 0.998 showed the stable linear detector response in different concentration range.

The proposed method was validated as per ICH guidelines. The method exhibited good selectivity and sensitivity. Percent recoveries for OFL (99.9-101.3%), ORD (99.9-100.7%), CLP (100-101.4%), TFH (99.8-100.7%), MP (99-101.8%), PP (100-100.9%).LOD and LOQ values indicate high sensitivity of the proposed method. The %RSD values of less than 2 for intra and inter day variation studies indicated that the proposed was precise. The developed method was studied for percentage recovery at three concentration levels and

%RSD values of less than 2 were found which were in acceptable limits indicates the method was accurate. Low %RSD values of less than 2 in variation of flow rate, wave length and mobile phase ratio indicates the method was robust. When the method was performed by two different analysts under the same experimental and environmental conditions and %RSD was found to be less than 2 indicating the ruggedness of the proposed method.

The results from solution stability experiments confirmed that sample was stable up to 24 hr. during assay determination. The sample recoveries of OFL, ORD, CLP, TFH, MP and PP from the commercial ointment dosage form were in good agreement with respective label claim indicating that there were no interferences from the commonly used excipients and buffer used in analysis.

**TABLE 1: PRECISION OF DEVELOPED METHOD**

| | METHOD PRECISION | | | | INTERDAY PRECISION | | | |
|---|---|---|---|---|---|---|---|---|
| **S.No** | **OFL** | | **ORD** | | **OFL** | | **ORD** | |
| | **RT** | **Area** | **RT** | **Area** | **RT** | **Area** | **RT** | **Area** |
| 1 | 0.718 | 4668006 | 1.763 | 6571429 | 0.702 | 4176605 | 1.910 | 5780438 |
| 2 | 0.717 | 4610096 | 1.762 | 6540887 | 0.702 | 4244423 | 1.906 | 5871623 |
| 3 | 0.715 | 4571376 | 1.745 | 6427233 | 0.699 | 4170094 | 1.904 | 5767214 |
| 4 | 0.718 | 4471493 | 1.763 | 6326667 | 0.701 | 4168076 | 1.907 | 5765667 |
| 5 | 0.717 | 4543521 | 1.753 | 6462119 | 0.704 | 4255283 | 1.907 | 5875064 |
| 6 | 0.719 | 4613446 | 1.770 | 6556781 | 0.707 | 4211807 | 1.910 | 5805109 |
| Mean | | 4579656 | | 6480853 | | 4204381 | | 5810853 |
| ±SD | | 0.141 | | 0.126 | | 0.082 | | 0.219 |
| % RSD | | 0.14 | | 0.13 | | 0.08 | | 0.22 |

| | METHOD PRECISION | | | | INTERDAY PRECISION | | | |
|---|---|---|---|---|---|---|---|---|
| **S.No** | **CLP** | | **TFH** | | **CLP** | | **TFH** | |
| | **RT** | **Area** | **RT** | **Area** | **RT** | **Area** | **RT** | **Area** |
| 1 | 9.713 | 326852 | 7.317 | 3822343 | 9.184 | 264766 | 7.277 | 3275140 |
| 2 | 9.703 | 325167 | 7.311 | 3802658 | 9.186 | 270259 | 7.267 | 3322543 |
| 3 | 9.681 | 356316 | 7.286 | 3900711 | 9.190 | 264838 | 7.280 | 3269665 |
| 4 | 9.687 | 325110 | 7.282 | 3935832 | 9.182 | 263739 | 7.274 | 3265499 |
| 5 | 9.735 | 326092 | 7.318 | 3880806 | 9.191 | 269729 | 7.280 | 3333311 |
| 6 | 9.696 | 327054 | 7.283 | 3894305 | 9.191 | 267002 | 7.280 | 3243003 |
| Mean | | 331098 | | 3872776 | | 266722 | | 3284860 |
| ±SD | | 0.699 | | 0.121 | | 1.082 | | 0.234 |
| % RSD | | 0.7 | | 0.12 | | 1.08 | | 0.23 |

| | METHOD PRECISION | | | | INTERDAY PRECISION | | | |
|---|---|---|---|---|---|---|---|---|
| **S.No** | **MP** | | **PP** | | **MP** | | **PP** | |
| | **RT** | **Area** | **RT** | **Area** | **RT** | **Area** | **RT** | **Area** |
| 1 | 4.177 | 5196486 | 7.996 | 474319 | 3.927 | 4772412 | 7.881 | 577887 |
| 2 | 4.178 | 5170506 | 7.990 | 469354 | 3.923 | 4843170 | 7.882 | 587880 |
| 3 | 4.113 | 5071282 | 7.970 | 472595 | 3.923 | 4757848 | 7.884 | 575943 |
| 4 | 4.242 | 4984933 | 9.986 | 461749 | 3.923 | 4755849 | 7.879 | 576470 |
| 5 | 4.234 | 5059011 | 8.029 | 485646 | 3.926 | 4855794 | 7.887 | 588184 |
| 6 | 4.323 | 5148416 | 8.00 | 472302 | 3.929 | 4799092 | 7.885 | 580113 |
| Mean | | 5105106 | | 4797361 | | 4797361 | | 581080 |
| ±SD | | 0.16 | | 0.316 | | 0.138 | | 0.344 |
| % RSD | | 0.16 | | 0.32 | | 0.14 | | 0.34 |

**TABLE 2: ACCURACY DATA**

| **% Level of recovery** | **Area** | **Amount of sample added (µg/ml)** | **Amount of API added (µg/ml)** | **Amount found (µg/ml±SD)** | **Recovery %±SD** | **%RSD** |
|---|---|---|---|---|---|---|
| | | **OFL** | | | | |
| 50% | 1997309 | 740 | 380 | 1120 | 100.8 | |
| | 2007584 | 740 | 380 | 1120 | 101.3 | 0.52 |
| | 1987413 | 740 | 380 | 1120 | 100.3 | |
| 100% | 3822894 | 740 | 740 | 1480 | 100.4 | |
| | 3856737 | 740 | 740 | 1480 | 99.9 | 0.310 |
| | 4168559 | 740 | 740 | 1480 | 99.9 | |
| 150% | 5753981 | 740 | 1100 | 1840 | 100.3 | |
| | 5674962 | 740 | 1080 | 1820 | 100.6 | 0.250 |
| | 5802068 | 740 | 1100 | 1840 | 100.2 | |
| | | **ORD** | | | | |
| 50% | 2713513 | 1950 | 970 | 2920 | 100.7 | |
| | 2746435 | 1950 | 975 | 2925 | 99.9 | 0.410 |
| | 2706202 | 1950 | 980 | 2930 | 100.4 | |
| 100% | 5443435 | 1950 | 1950 | 3900 | 100.5 | |
| | 5446624 | 1950 | 1950 | 3900 | 100.0 | 0.23 |
| | 5489990 | 1950 | 1950 | 3900 | 100.3 | |
| 150% | 8812268 | 1950 | 3160 | 5110 | 100.4 | |
| | 8749680 | 1950 | 3100 | 5050 | 100.3 | 0.050 |
| | 8973843 | 1950 | 3150 | 5100 | 100.3 | |
| | | **CLP** | | | | |
| 50% | 116679 | 420 | 210 | 630 | 100.0 | |
| | 118193 | 420 | 205 | 625 | 100.0 | 0 |
| | 114709 | 420 | 211 | 631 | 100.0 | |

| | | | | | | |
|---|---|---|---|---|---|---|
| 100% | 234724 | 420 | 419 | 839 | 100.0 | |
| | 238125 | 420 | 420 | 840 | 100.0 | 0 |
| | 241984 | 420 | 421 | 841 | 101.4 | |
| 150% | 395580 | 420 | 630 | 1050 | 100.0 | |
| | 391541 | 420 | 631 | 1051 | 101.2 | 0.77 |
| | 478105 | 420 | 632 | 1052 | 100.3 | |

| **% Level of recovery** | **Area** | **Amount of sample added (µg/ml)** | **Amount of API added (µg/ml)** | **Amount found (µg/ml±SD)** | **Recovery %±SD** | **%RSD** |
|---|---|---|---|---|---|---|
| | | **TFH** | | | | |
| 50% | 1533872 | 730 | 400 | 1130 | 100.5 | |
| | 1528082 | 730 | 390 | 1120 | 100.1 | 0.320 |
| | 1510038 | 730 | 410 | 1140 | 100.7 | |
| 100% | 2779055 | 730 | 729 | 1459 | 99.8 | |
| | 2768592 | 730 | 730 | 1460 | 99.9 | 0.150 |
| | 2848790 | 730 | 730 | 1460 | 100.1 | |
| 150% | 4697022 | 730 | 1100 | 1830 | 100.0 | |
| | 4670484 | 730 | 1080 | 1810 | 100.2 | 0.200 |
| | 4752992 | 730 | 1100 | 1830 | 100.4 | |
| | | **MP** | | | | |
| 50% | 2209976 | 200 | 110 | 310 | 101.0 | |
| | 2436773 | 200 | 100 | 300 | 101.8 | 0.470 |
| | 2205330 | 200 | 110 | 310 | 101.0 | |
| 100% | 4335272 | 200 | 200 | 400 | 99.5 | |
| | 4333110 | 200 | 210 | 410 | 99.0 | 0.77 |
| | 4393648 | 200 | 200 | 400 | 100.5 | |
| 150% | 7039035 | 200 | 300 | 500 | 100.6 | |
| | 7008323 | 200 | 310 | 510 | 100.3 | 0.470 |
| | 7192234 | 200 | 300 | 500 | 99.7 | |
| | | **PP** | | | | |
| 50% | 220691 | 20 | 10 | 30 | 100.0 | |
| | 220789 | 20 | 11 | 31 | 100.9 | 0.530 |
| | 217683 | 20 | 10 | 30 | 100.9 | |
| 100% | 423475 | 20 | 20 | 40 | 100.0 | |
| | 426509 | 20 | 21 | 41 | 100.5 | 0.280 |
| | 434890 | 20 | 22 | 42 | 100.5 | |
| 150% | 647841 | 20 | 30 | 50 | 100.3 | |
| | 640313 | 20 | 31 | 51 | 100.0 | 0.310 |
| | 652103 | 20 | 32 | 52 | 100.6 | |

**TABLE 3: VALIDATION AND SYSTEMSUITABILITY PARAMETERS**

| PARAMETER | OFL | ORD | CLP | TFH | MP | PP |
|---|---|---|---|---|---|---|
| Range (µg/ml) | 8 -96 | 80 -960 | 1 -20 | 29 -348 | 8 -96 | 1 -9.72 |
| Slope | 48218 | 7050 | 15443 | 9726 | 57239 | 56780 |
| Intercept | 91029 | 24359 | 7584 | 23790 | 28957 | 3783 |
| Correlation coefficient ($R^2$) | 0.999 | 0.999 | 0.998 | 0.999 | 0.998 | 0.9997 |
| Retention time | 0.712 | 1.933 | 9.224 | 7.302 | 4.074 | 7.926 |
| Precision (intra and inter day)% RSD | <2 | <2 | <2 | <2 | <2 | <2 |
| Accuracy | 99.9 - 101.3 | 99.9 - 100.7 | 100 - 101.4 | 99.8 - 100.7 | 99.0 - 101.8 | 100.0 - 100.9 |
| LOD(µg/ml) | 1.83 | 9.52 | 0.566 | 4.37 | 1.66 | 0.123 |
| LOQ(µg/ml) | 5.57 | 28.8 | 1.717 | 13.26 | 5.035 | 0.374 |
| Tailing factor | 1.08 | 1.07 | 1.06 | 1.18 | 1.09 | 1.15 |
| Theoretical plates | 833 | 1077 | 85275 | 44830 | 3361 | 54056 |
| Resolution | - | 7.29 | 9.66 | 15.07 | 8.18 | 4.44 |

**TABLE 4: INFLUENCE OF FLOW RATE, WAVELENGTH AND MOBILE COMPOSITION ON ANALYTICAL PARAMETERS**

| Parameter | 0FL | | | ORD | | |
|---|---|---|---|---|---|---|
| | RT | Area | Tailing | RT | Area | Tailing |
| **Flow rate(±0.2ml/min)** | | | | | | |
| 0.8 | 0.886 | 5378272 | 1.15 | 2.31 | 8439366 | 1.23 |
| 1 | 0.888 | 4444563 | 1.17 | 2.31 | 6948836 | 1.28 |
| 1.2 | 0.615 | 3885043 | 1.35 | 1.53 | 5702432 | 1.25 |
| **Wave length(±5nm)** | | | | | | |
| 250 | 0.69 | 4166848 | 1.08 | 1.90 | 5925415 | 1.07 |
| 255 | 0.70 | 4357204 | 1.07 | 1.92 | 6054390 | 1.06 |
| 260 | 0.69 | 3690713 | 1.09 | 1.90 | 5911387 | 1.07 |
| **Mobile phase composition (±5%v/v)** | | | | | | |
| 13:87 | 0.75 | 4190353 | 1.14 | 2.15 | 7160699 | 1.22 |
| 18:82 | 0.68 | 3960273 | 1.32 | 1.56 | 5554167 | 1.34 |
| 23:77 | 0.68 | 4765060 | 1.27 | 1.58 | 6697843 | 1.26 |

| Parameter | CLP | | | TFH | | |
|---|---|---|---|---|---|---|
| | RT | Area | Tailing | RT | Area | Tailing |
| **Flow rate(±0.2ml/min)** | | | | | | |
| 0.8 | 11.3 | 425058 | 1.21 | 8.72 | 4774015 | 1.43 |
| 1 | 11.3 | 306456 | 1.20 | 8.78 | 3497545 | 1.37 |
| 1.2 | 8.60 | 311870 | 1.18 | 6.30 | 3295554 | 1.47 |
| **Wave length(±5nm)** | | | | | | |
| 250 | 9.18 | 330666 | 1.08 | 7.27 | 5735624 | 1.17 |
| 255 | 9.21 | 332366 | 1.08 | 7.29 | 5612004 | 1.18 |
| 260 | 9.18 | 216823 | 1.06 | 7.27 | 3028708 | 1.17 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Mobile phase composition** | | | | **(±5%v/v)** | | |
| 13:87 | 9.90 | 355878 | 1.17 | 7.45 | 3940594 | 1.43 |
| 18:82 | 9.53 | 256300 | 1.19 | 7.07 | 2854477 | 1.39 |
| 23:77 | 9.43 | 347157 | 1.17 | 7.05 | 3969105 | 1.54 |

| **Parameter** | **MP** | | | **PP** | | |
|---|---|---|---|---|---|---|
| | **RT** | **Area** | **Tailing** | **RT** | **Area** | **Tailing** |
| **Flow rate (±0.2ml/min)** | | | | | | |
| 0.8 | 5.57 | 6598819 | 1.18 | 9.57 | 602582 | 1.22 |
| 1 | 5.56 | 5537080 | 1.20 | 9.60 | 537696 | 1.22 |
| 1.2 | 3.61 | 4520476 | 1.20 | 6.94 | 409664 | 1.19 |
| **Wave length (±5nm)** | | | | | | |
| 250 | 3.92 | 4308864 | 1.10 | 7.88 | 524094 | 1.16 |
| 255 | 4.05 | 4402242 | 1.09 | 7.91 | 531981 | 1.15 |
| 260 | 3.92 | 4561884 | 1.10 | 7.88 | 548739 | 1.16 |
| **Mobile phase composition** | | | | **(±5%v/v)** | | |
| 13:87 | 5.55 | 5571100 | 1.13 | 8.25 | 500453 | 1.22 |
| 18:82 | 3.47 | 4522014 | 1.25 | 7.74 | 443906 | 1.18 |
| 23:77 | 3.50 | 5402192 | 1.22 | 7.74 | 496287 | 1.21 |

**TABLE 5: ASSAY OF COMMERCIAL FORMULATION**

| **Drug** | **Label claim(mg/tablet)** | **Calculated value (ml±SD/tablet)** | **% of Assay** |
|---|---|---|---|
| OFL | 0.75 | 0.76 | 101.3% |
| ORD | 2 | 1.99 | 99.5% |
| CLP | 0.05 | 0.0510 | 102 % |
| TFH | 1 | 0.98 | 98 % |
| MP | 0.2 | 0.201 | 100% |
| PP | 0.02 | 0.0202 | 101% |

**TABLE 6: Stability Studies**

| **S.NO** | **Drug** | **Stability** | **RT** | **Area** | **USP Tailing** | **USP Plate count** |
|---|---|---|---|---|---|---|
| 1 | OFL | | 0.701 | 4228339 | 1.16 | 835 |
| 2 | ORD | | 1.907 | 5840453 | 1.08 | 1080 |
| 3 | CLP | 24hrs | 9.183 | 268832 | 1.07 | 83292 |
| 4 | TFH | | 7.271 | 3285903 | 1.20 | 42122 |
| 5 | MP | | 3.925 | 4808694 | 1.10 | 3270 |
| 6 | PP | | 7.879 | 583001 | 1.15 | 54822 |
| **S.NO** | **Drug** | | **RT** | **Area** | **USP Tailing** | **USP Plate count** |
| 1 | OFL | | 0.703 | 3969525 | 1.12 | 858 |
| 2 | ORD | 12 hrs | 1.913 | 5792163 | 1.07 | 1095 |
| 3 | CLP | | 9.183 | 265519 | 1.05 | 83316 |
| 4 | TFH | | 7.273 | 3236684 | 1.20 | 42301 |
| 5 | MP | | 3.946 | 4764552 | 1.10 | 3309 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 | PP | | 7.882 | 578147 | 1.15 | 55162 |
| **S.NO** | **Drug** | | **RT** | **Area** | **USP Tailing** | **USP Plate count** |
| 1 | OFL | | 0.708 | 4028958 | 1.07 | 841 |
| 2 | ORD | | 1.925 | 5805037 | 1.07 | 1108 |
| 3 | CLP | 0 hrs | 9.203 | 261599 | 1.07 | 85588 |
| 4 | TFH | | 7.291 | 3179164 | 1.16 | 42561 |
| 5 | MP | | 4.003 | 4757799 | 1.10 | 3353 |
| 6 | PP | | 7.905 | 573931 | 1.15 | 56341 |

**TABLE 7: Forced Degradation studies**

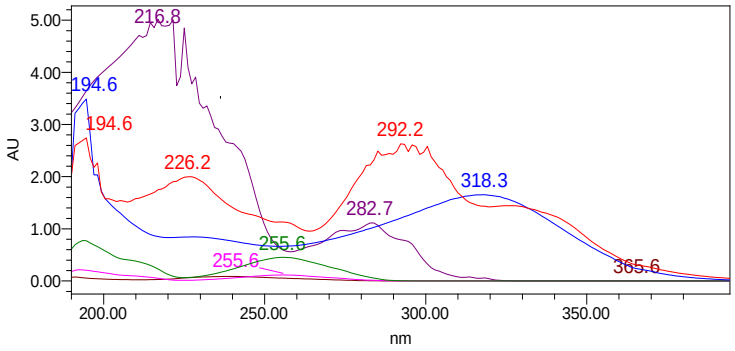
| **Parameter** | **Sample wt(mg)** | | **Area counts** | | **%label claim** | | **%degradation** | |
|---|---|---|---|---|---|---|---|---|
| | **ORD** | **OFL** | **ORD** | **OFL** | **ORD** | **OFL** | **ORD** | **OFL** |
| Control | 9682 | 982.2 | 3421249 | 3421249 | 10% | 100% | 90 | 0 |
| Acid | 895.1 | 907.5 | 2530272 | 2530272 | 80.1 | 80 | 19.9 | 20 |
| Alkali | 891.7 | 905.4 | 2520729 | 2520729 | 80.1 | 79.9 | 19.9 | 20.1 |
| Peroxide | 962.8 | 978.2 | 2722021 | 2722021 | 80.1 | 79.9 | 19.9 | 20.1 |
| Reduction | 1108.1 | 1124.1 | 3125535 | 3125535 | 79.9 | 79.8 | 20.1 | 20.2 |
| Thermal | 906.3 | 915 | 2547245 | 2547245 | 79.6 | 79.9 | 20.4 | 20.1 |
| Photolytic | 1079.5 | 1088.3 | 3037092 | 3037092 | 79.7 | 80.1 | 20.3 | 19.9 |
| Humidity | 1095.6 | 1109.5 | 3083741 | 3083741 | 79.7 | 79.8 | 20.3 | 20.2 |
| Hydrolysis | 1110.2 | 1130.4 | 3144375 | 3144375 | 80.2 | 79.9 | 19.8 | 20.1 |
| Heat | 1115.5 | 1128.5 | 3144375 | 3144375 | 79.8 | 80 | 20.2 | 20 |

| **Parameter** | **Sample wt(mg)** | | **Area counts** | | **%label claim** | | **%degradation** | |
|---|---|---|---|---|---|---|---|---|
| | **CLP** | **TFH** | **CLP** | **TFH** | **CLP** | **TFH** | **CLP** | **TFH** |
| Control | 822.2 | 728.1 | 3421249 | 3421252 | 100 | 99.6 | 0 | 0.4 |
| Acid | 760.1 | 682.2 | 2530272 | 2530272 | 80 | 78.5 | 20 | 21.5 |
| Alkali | 759.2 | 686.3 | 2520729 | 2520729 | 79.8 | 77.8 | 20.2 | 22.2 |
| Peroxide | 820.2 | 745.6 | 2722021 | 2722021 | 79.8 | 77.3 | 20.2 | 22.7 |
| Reduction | 945.2 | 844.5 | 3125535 | 3125535 | 79.5 | 78.4 | 20.5 | 21.6 |
| Thermal | 768.1 | 685.2 | 2547245 | 2547245 | 79.7 | 78.7 | 20.3 | 21.3 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Photolytic | 915.6 | 810.3 | 3037092 | 3037092 | 79.7 | 79.4 | 20.3 | 20.6 |
| Humidity | 928.2 | 816.3 | 3083741 | 3083741 | 79.9 | 80 | 20.1 | 20 |
| Hydrolysis | 961.2 | 838.6 | 3144375 | 3144375 | 78.6 | 79.4 | 21.4 | 20.6 |
| Heat | 981.2 | 832.6 | 3144375 | 3144375 | 77 | 80 | 23 | 20 |

| **Parameter** | **Sample wt(mg)** | | **Area counts** | | **%label claim** | | **%degradation** | |
|---|---|---|---|---|---|---|---|---|
| | **MP** | **PP** | **MP** | **PP** | **MP** | **PP** | **MP** | **PP** |
| Control | 979.2 | 1027.9 | 3421249 | 3421252 | 100.1 | 100 | -0.1 | 0 |
| Acid | 905.5 | 1027.9 | 2530272 | 2530272 | 80.1 | 74 | 19.9 | 26 |
| Alkali | 903.7 | 1027.9 | 2520729 | 2520729 | 79.9 | 73.7 | 20.1 | 26.3 |
| Peroxide | 970.4 | 1027.9 | 2722021 | 2722021 | 80.4 | 79.6 | 19.6 | 20.4 |
| Reduction | 1120.1 | 1217.9 | 3125535 | 3125535 | 79.9 | 77.1 | 20.1 | 22.9 |
| Thermal | 915.2 | 1027.9 | 2547245 | 2547245 | 79.7 | 74.5 | 20.3 | 25.5 |
| Photolytic | 1090.5 | 11545.9 | 3037092 | 3037092 | 79.8 | 79.6 | 20.2 | 20.4 |
| Humidity | 1102.6 | 1155.9 | 3083741 | 3083741 | 80.1 | 80.5 | 19.2 | 19.8 |
| Hydrolysis | 1124.2 | 1215.9 | 3144375 | 3144375 | 80.1 | 77.7 | 19.9 | 22.3 |
| Heat | 1127.3 | 1217.9 | 3144375 | 3144375 | 79.9 | 77.6 | 20.1 | 22.4 |

**Figure 1: Overlay UV Spectra of Standard OFL, ORD, CLP, TFH, MP and PP**




---------- **Ofloxacin**
---------- **Ornidazole**
---------- **Methyl paraben**
---------- **Terbinafine Hcl**
---------- **Propyl paraben**
---------- **Clobetasol propionate**

**Figure 2: Typical HPLC chromatogram of OFL, ORD, CLP, TFH, MP and PP**

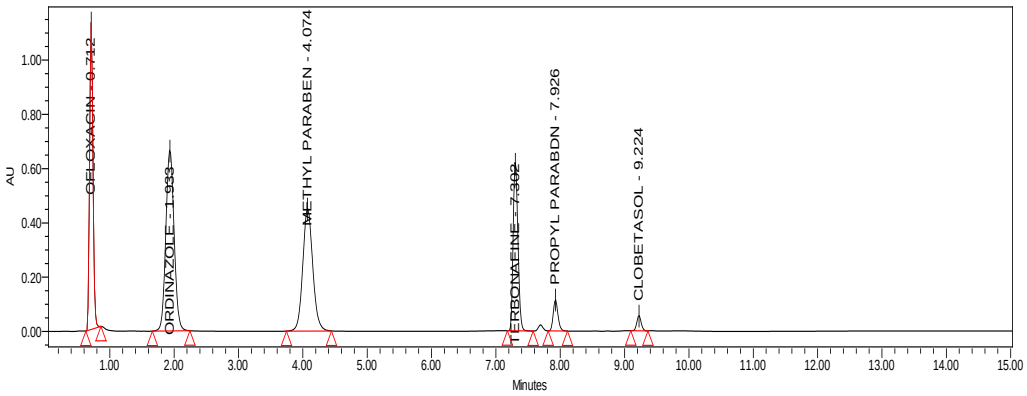



**Figure 3: Individual chromatogram of Ofloxacin**

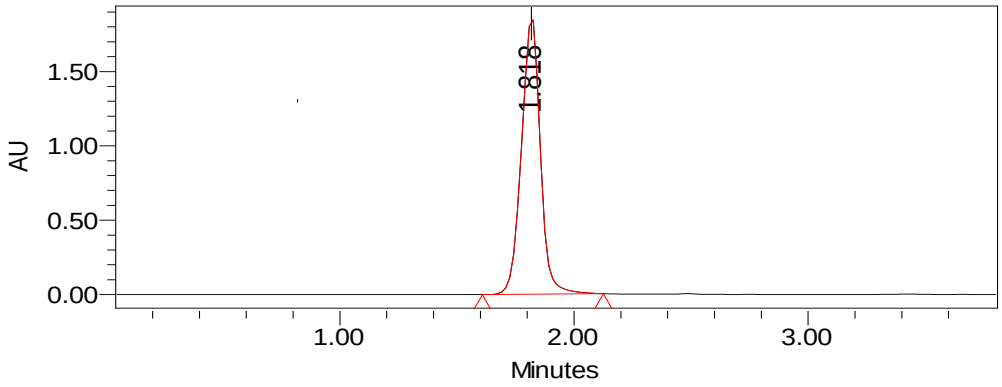



**Figure 4: Individual chromatogram of Ornidazole**

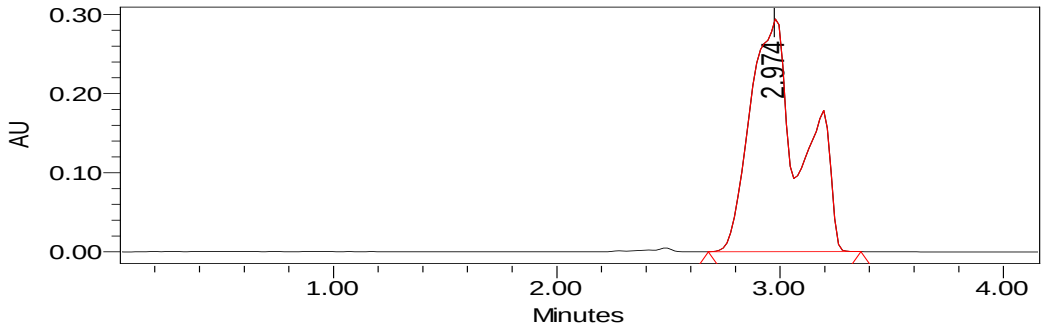


**Figure 5: Individual chromatogram of Clobetasol propionate**

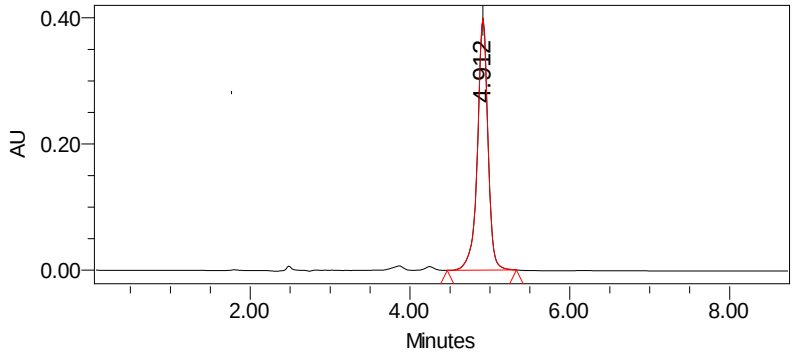



**Figure 6: Individual chromatogram of Terbinafine Hcl**

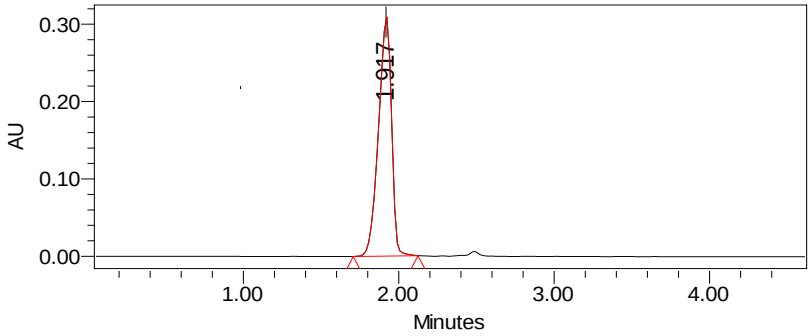



**Figure 7: Individual chromatogram of Methyl paraben**

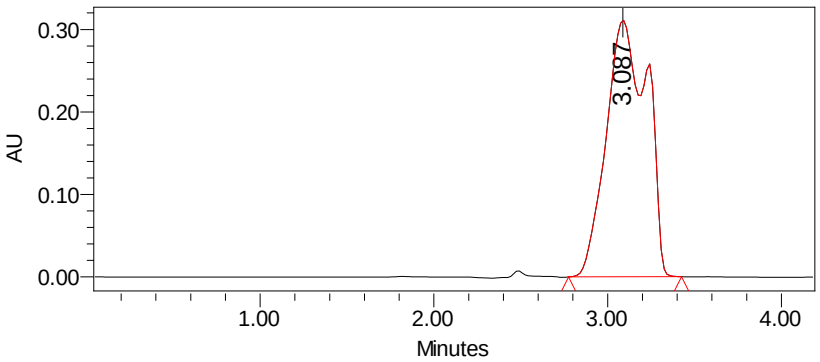


**Figure 8: Individual chromatogram of Propyl paraben**

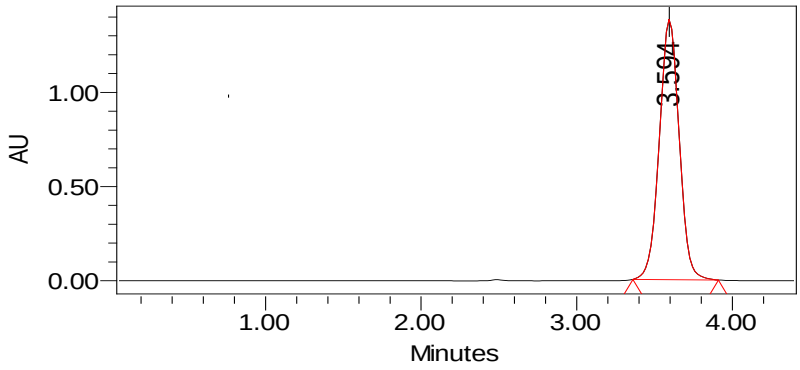



**Figure 9: calibration curve for Ofloxacin**

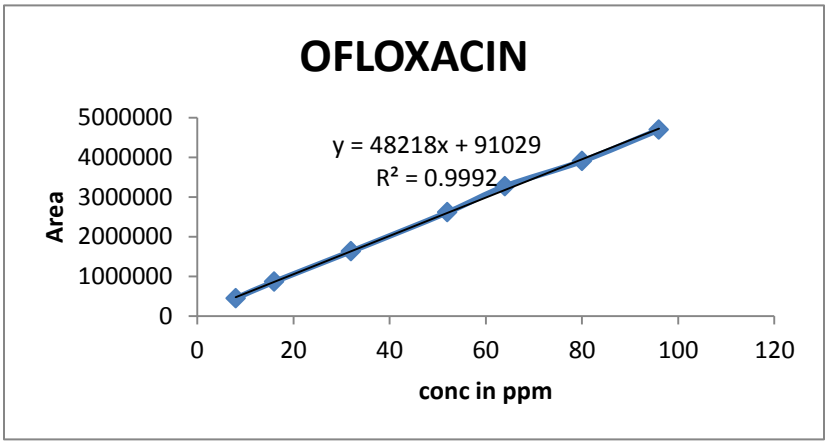



**Figure 10: calibration curve for Ornidazole**

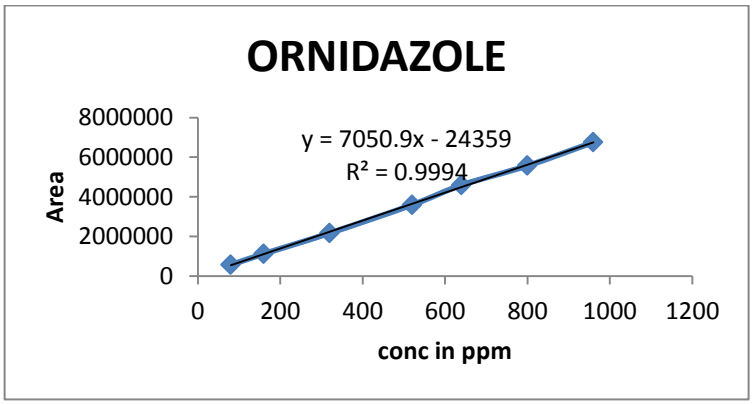


**Figure 11: calibration curve for Clobetasol propionate**

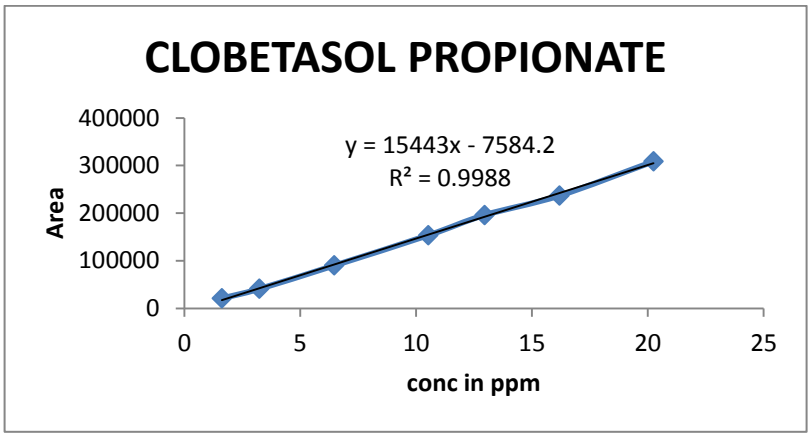



**Figure 12: calibration curve for Terbinafine Hcl**

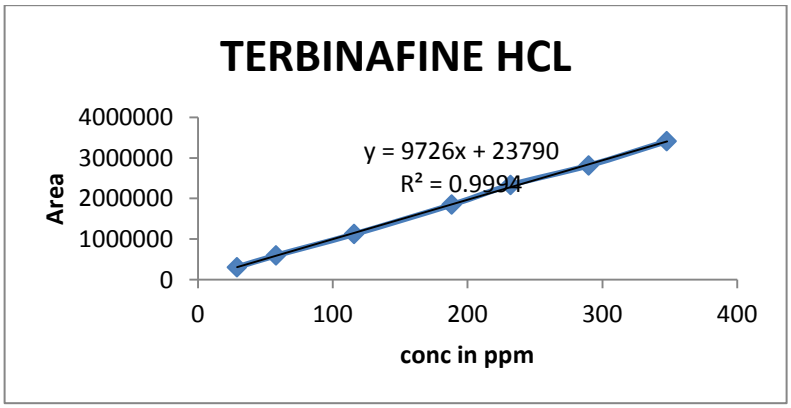



**Figure 13: Calibration curve for Methyl paraben**

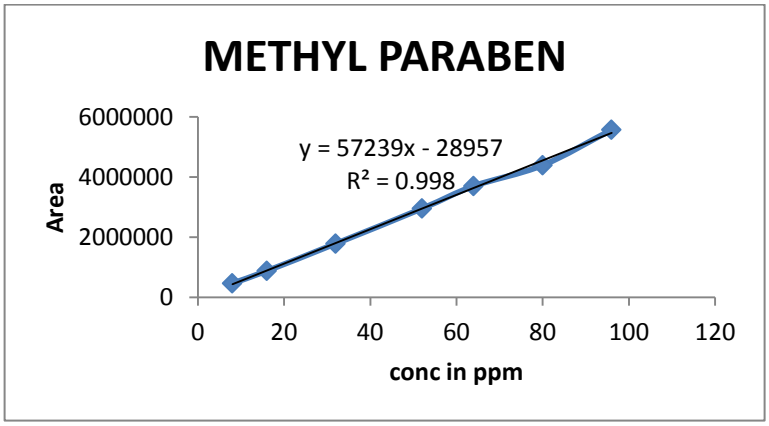
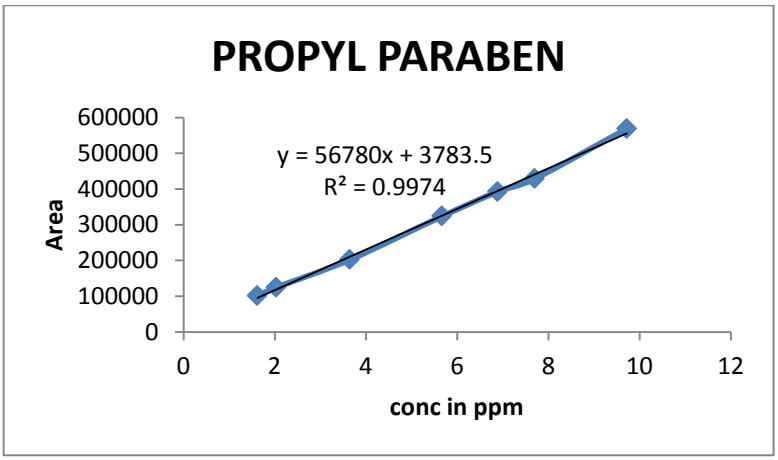



**Figure14: calibration curve for Propyl paraben**




## CONCLUSION

The low standard deviation and %RSD calculated for the proposed developed method and validation were in conformity with standards. The results of stress testing under taken according to the ICH guidelines reveal that the method is specific and stability indicating. Hence, it can be concluded that the developed RP-HPLC method is accurate, precise and selective and can be employed successfully for the simultaneous estimation of OFL, ORD, CLP, TFH, MP and PP in ointment dosage form for routine quality control analysis.

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