Development and Validation of Area under Curve Method for Simultaneous Estimation of Clobetasol Propionate and Fusidic acid in Cream

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Abstract: **A simple, specific, accurate and precise Area Under Curve spectrophotometric method was developed and validated for simultaneous estimation of Clobetasol propionate and Fusidic acid in cream. The linearity range was found to be 0.5- 2.5 µg/ml for Clobetasol propionate and 20-100 µg/ml for Fusidic acid. The co-relation co-efficient was found to be 0.9983, 0.9992 and 0.9989, 0.9983 for Clobetasol propionate and Fusidic acid at λ_1 233.00 -241.00 nm and λ_2 220.00 – 228.00 nm respectively. The % recovery were found to be 99.71- 100.57, 99.38- 100.45 and 99.65- 100.51, 98.648-99.859 for Clobetasol propionate and Fusidic acid at λ_1 233.00 -241.00 nm and λ_2 220.00 – 228.00 nm respectively. Intraday precision were found to be 0.11-0.13, 0.19-0.24 and 0.29-0.31, 0.21-0.27 % RSD for Clobetasol propionate and Fusidic acid at λ_1 233.00 -241.00 nm and λ_2 220.00 – 228.00 nm and Interday precision were found to be within the range.**

Keywords: **Clobetasol propionate, Fusidic acid, Area Under Curve, UV Spectroscopy method, Validation.**

1. INTRODUCTION:

Clobetasol propionate (21-Chloro-9-fluoro-11β-hydroxy-16βmethyl-3, 20-dioxopregna-1,4-dien-17-yl propanoate) is derivative of prednisolone with high glucocorticoid activity and low mineralocorticoid activity, Fusidic acid [ent-(17Z)-16α- (Acetyloxy)-3β, 11β-dihydroxy-4β, 8, 14- trimethyl-18-nor-5β, 10α-cholesta-17(20), 24-dien21-oic acid hemihydrate] is an antimicrobial substance. It is reported in latest pharmacopoeias such as IP 2018 BP 2016 and USP 2017[1-3] .Combination is approved by CDSCO on **17/07/2015**.

Several analytical methods like UV[5,6],HPLC[7-9], and HPTLC are reported alone and in combination with other drugs for the determination of Clobetasol propionate and Fusidic acid in the literature for its assay. However there is no method reported for simultation estimation of Clobetasol propionate and Fusidic acid by UV spectroscopy in any literature. Therefore, I have developed Area under Curve Spectroscopic method for the combination of Clobetasol Propionate and Fusidic acid in cream and validation of developed methods as per ICH guidelines **(Figure 1 and 2)**.

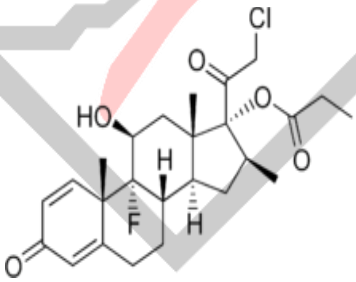

Figure 1: Structure of Clobetasol propionate

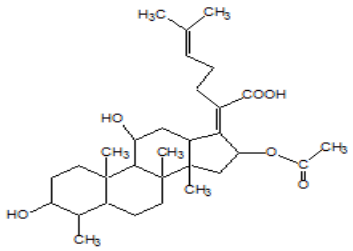

Figure 2: Structure of Fusidic acid

Area under Curve Method:

In case of AUC (Area under Curve) method is applicable where there is no sharp peak or broad spectra is obtained. In that involves the calculation of integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ1 and λ2. The Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by the entering the wavelength ranges over which area has to be calculated. This wavelength range is selected on the basis of repeated observation so as to get the linearity between area under curve and concentration. The above mentioned spectrums were used to calculate AUC. Thus, the calibration curve can be constructed by plotting concentration versus AUC.[12]

2. MATERIALS AND METHODS

2.1. Instrumentation:

Double beam UV-Visible Spectrophotometer Shimadzu-1800, software: - UV probe (v-2.42).

2.2 Reagents and materials

All the reagents and solvents used were of AR grades. Clobetasol propionate was obtained as gift sample from Avik Pharmaceutical Ltd, Vapi and Fusidic acid was obtained as a gift sample from Aroma Remedies Daman.Marketed formulation LOZIVATE®-F (Clobetasol propionate 0.05%W/W, Fusidic acid 2.0 %W/W) tablet Manufacture by Canixa Life Science Pvt, UTTARAKHAND

2.3. Preparation of standard stock solution of Clobetasol Propionate (CP).

12.5 mg of was weighed and transferred to 25 ml volumetric flask. Make final volume with methanol (500 µg/ml). Pipette out 1ml in 10 ml of volumetric flask containing methanol (50 µg/ml).From above solution pipette out 0.1, 0.2,0.3,0.4 and 0.5 ml in 10 ml of volumetric flask to make up the final volume with methanol to get final concentration range of (0.5-2.5 µg/ml).

2.4. Preparation of standard stock solution of Fusidic acid (FA).

50 mg of FA was weighed and transferred to 25 ml volumetric flask. It was dissolved in methanol and volume was made up to the mark with methanol to give a solution containing (2000 µg/ml). Aliquot of 2 ml from above standard stock solution was pipette out into 10 ml of volumetric flask and volume was made up to the mark with methanol to give a solution containing (400 µg/ml). From above solution pipette out 0.5, 1, 1.5,2 and 2.5 ml in 10 ml of volumetric flask to make the final concentration range of (20-100 µg/ml).

2.5. Preparation of standard stock solution of methyl paraben (MP).

4 mg of MP was weighed and transferred to 10 ml volumetric flask. It was dissolved in methanol and volume was made up to the mark with methanol to give a solution containing (400 µg/ml). Aliquot of 1 ml from above standard stock solution was pipette out into 10 ml of volumetric flask and volume was made up to the mark with methanol to give a solution containing (40 µg/ml). From above solution pipette out 0.5, 1, 1.5,2 and 2.5 ml in 10 ml of volumetric flask to make the final concentration range of (2-10 µg/ml).

2.5. Selection of analytical wavelength range for CP , FA and MP for AUC Method[10]

Appropriate dilution were prepared for CP , FA and MP from the standard stock solution and the solution were scanned in the wavelength range of 200-400 nm. The absorption spectra obtained were showing the absorption maxima [λ max] at 237 nm, 225nm and 250nm and Area Under Curve [AUC] in absorption spectra were measured between the wavelength range 233.00 to 241.00 nm 220.00 to 228.00 nm and 250 to 258 nm where good linear area was found in selected concentration range.

2.6. VALIDATION OF PROPOSED METHOD

2.6.1. Linearity (n=5)

The linearity response was determined by analyzing 5 independent levels of calibration curve in the range of 0.5-2.5 µg/ml for CP , 20-100 µg/ml for FA and 20-10 µg/ml for MP (n=5).

2.6.2. Precision

2.6.2.1. Repeatability

Prepare 2 µg/ml of CP, 80 µg/ml of FA and 8 µg/ml of MP solution were analysed six times (n=6) by short interval time and % R.S.D. was calculated.

2.6.2.2. Intraday Precision

Prepere 1, 1.5 and 2µg/ml of CP , 40, 60 and 80 µg/ml of FA and 4 , 6 and 8 µg/ml of MP solution were analysed for three times (n=3) on the same day within short interval of time and % R.S.D. was calculated.

2.6.2.3. Interday Precision

Prepere 1, 1.5 and 2µg/ml of CP , 40, 60and 80 µg/ml of FA and 4 , 6 and 8 µg/ml of MP solution were analysed for three times (n=3) on the same day within short interval of time and % R.S.D. was calculated.

2.6.3. Accuracy

Accuracy study was performed by spike method. In this method, standard solution of drug was added to extracted solution of cream. Each solution was scanned from 200-400 nm against Distilled water as a blank. Absorbance of solution was measured at selected wavelengths for CP, FA and MP. The amount of CP, FA and MP was calculated at each level (80%, 100% and 120%) and % recoveries were found.

2.6.4. LOD and LOQ

The LOD (Limit of Detection) was estimated from the set of 5 calibration curves that were used to determine linearity of the method. The LOD was calculated by using the formula:

LOD = 3.3 × (S.D./Slope)

The LOQ (Limit of Quantitation) was estimated from the set of 5 calibration curves that were used to determine linearity of the method.

The LOQ was calculated by using the formula:

LOQ = 10 × (S.D./Slope)

2.6.5. Analysis of Marketed Formulation

2.6.5.1. Sample preparation

1gm of cream (Marketed Formulation) equivalent weight (0.5mg of CP, 20 mg FA and 4 mg MP) was taken into 50 ml of volumetric flask. Methanol was added and sonicated (60-65℃) for 10 mins and volume was made up to mark with methanol. Solution was filtered through Whatmann filter paper no. 42. Thus, resulting solution gave 10 µg/ml of CP , 400 µg/ml of FA and 40 µg/ml of MP. From the above solution, 1.0 ml was pipette out and transferred to 10 ml of volumetric flask and volume was made upto mark with methanol to give a solution containing CP (1 µg/ml) + FA (40 µg/ml) + MP (4 µg/ml) . This solution was used for assay i.e. estimation of CP, FA and MP in Marketed formulation.

2.6.5.2. Estimation of Clobetasol Propionate, Fusidic acid and Methyl Paraben [10]

Area of the resulting solution was measured at 233.00 to 241.00 nm , 220.00 to 228.00 nm and 250.00 to 258.00 nm against methanol. The concentration of CP, FA and MP can be obtained as,

A set of two simultaneous equations were established using mean absorptivity of coefficient of CP and FA at selected wavelength range interval.

$A_1 = ax_1C_1 + ay_1C_2$… ..... Eq (1) at 233.00 to 241.00 nm

$A_2 = ax_2C_2 + ay_2C_2$… ..... Eq (2) at 220.00 to 228.00 nm

$A_2 = ax_3C_3 + ay_3C_3$… ..... Eq (3) at 250.00 to 258.00 nm

Where,

$ax_1$and ax_2 are mean absorptivity of CP at AUC 233.00 to 241.00 nm ,220.00 to 228.00 nm and 250.00 to 258.00 nm respectively.

$ay_1$and ay_2 are mean absorptivity of FA at AUC 220.00 to 228.00 nm , 233.00 to 241.00 nm and 250.00 to 258.00 nm respectively.

ay_3 are mean absorptivity of MP at AUC 220.00 to 228.00 nm, 233.00 to 241.00 nm and 250.00 to 258.00 nm respectively.

C_1 , C_2 and C_3 are concentration (gm/L) of CP , FA and MP.

A_1 , A_2 and A_3 are sample solution at AUC 233.00 to 241.00 nm, 220.00 to 228.00 nm and 250.00 to 258.00 nm respectively. By applying the Cramer's rules to equation (1) and (2), the conc. of C_1 , C_2 and $C_3$are obtained.

3. RESULTS AND DISCUSSION

Overlain spectra of CP (0.5 µg/ml), FA (20 µg/ml) and MP (2 µg/ml) were taken. Overlain spectra were given in **(Figure. 3)**. Linearity for CP was 0.5 – 2.5 µg/ml, FA was 20 – 100 µg/ml and MP was 2 – 10 µg/ml were found. AUC spectra for drugs given in **(Figure. 4,5,6)** . Calibration curve was given at wavelength 233-241nm , wavelength 220-228 nm and wavelength 250-258 nm given in **(Figure.7 to 15)**. Linearity data were given in **(Table 1 to 12)**. Precision was performed as repeatability, intraday precision and interday precision. Repeatability data were given in **(Table 13)**. Intraday precision were given in **(Table 14)** . Interday precision data were given in **(Table 15)**. %R.S.D. limit was found less than 2. Acuracy data were given in **(Table 16,17, 18)** and LOD was found and LOQ was found. Assay was found 99.93-100.19%. Assay result was given in **(Table 18)**.

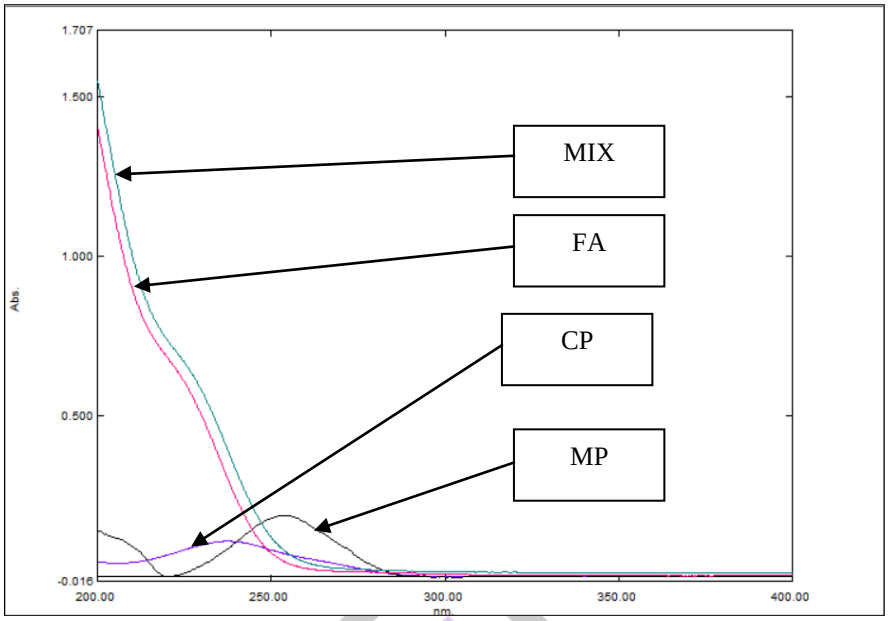

Figure 3: Overlain of CP (0.5-2.5µg/ml) and FA (20-100µg/ml) and MP (2 -10 µg/ml)Sample (0.5+20+2 µg/ml)

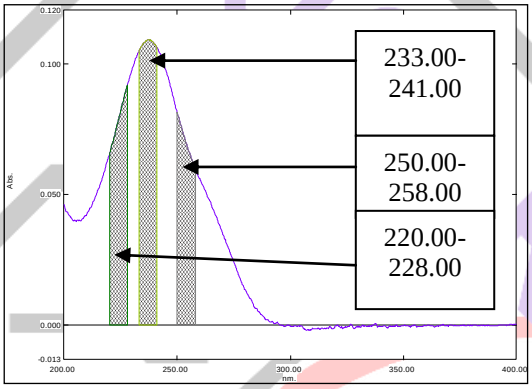

Figure 4: AUC Spectra of CP 0.5 µg/ml at wavelength 233.00 to 241.00 nm , 220.00 to 228.00 nm and 250.00 to 258.00 nm.

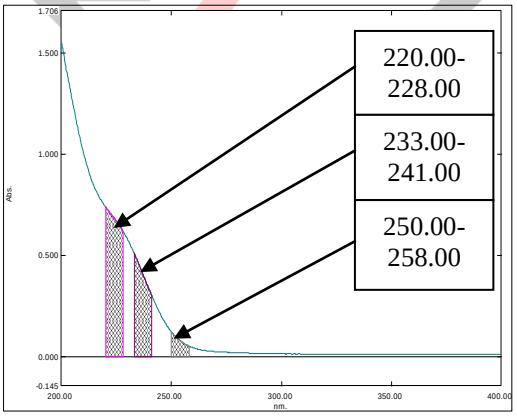

Figure 5: AUC Spectra of FA 20 µg/ml at wavelength 233.00 to 241.00 nm , 220.00 to 228.00 nm and 250.00 to 258.00 nm.

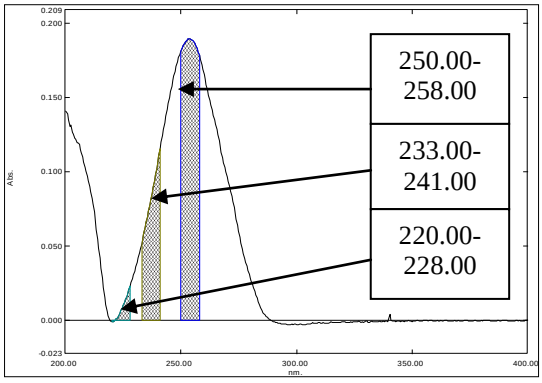

Figure 6: AUC Spectra of MP 2 µg/ml at wavelength 233.00 to 241.00 nm , 220.00 to 228.00 nm and 250.00 to 258.00 nm.

Method Validation:

1. Linearity

The linearity range for CP, FA and MP was found to be in the range of 0.5-2.5 µg/ml , 20-100 µg/ml and 2-10 µg/ml.

Table 1: Linearity data for CP at 233.00 - 241.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	0.5	0.860 ± 0.005381	0.626
2	1	1.114 ± 0.007092	0.636
3	1.5	1.363 ± 0.002302	0.168
4	2	1.693 ± 0.004393	0.259
5	2.5	1.987 ± 0.004939	0.248

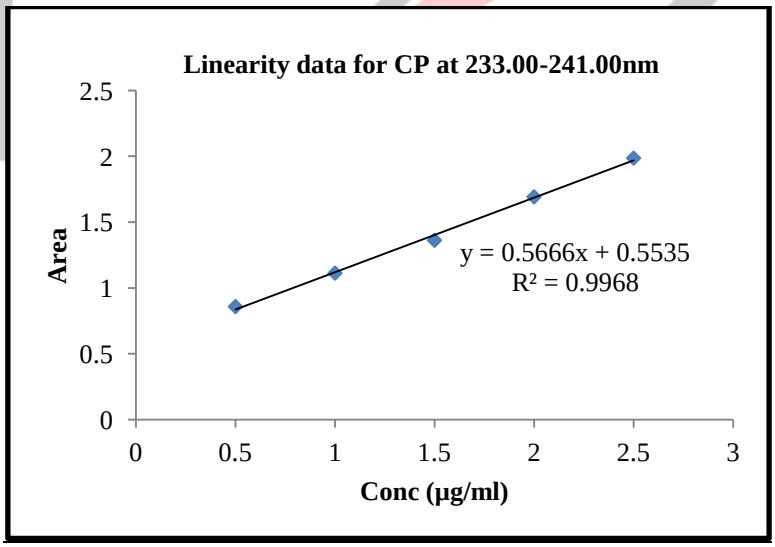

Figure 7: Calibration curve for CP at 233.00- 241.00 nm

Table 2: Linearity data for FA at 233.00-241.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	20	1.296 ± 0.001581	0.113
2	40	1.869 ± 0.001924	0.102
3	60	2.393 ± 0.003114	0.130
4	80	3.128 ± 0.003975	0.127
5	100	3.755 ± 0.015630	0.416

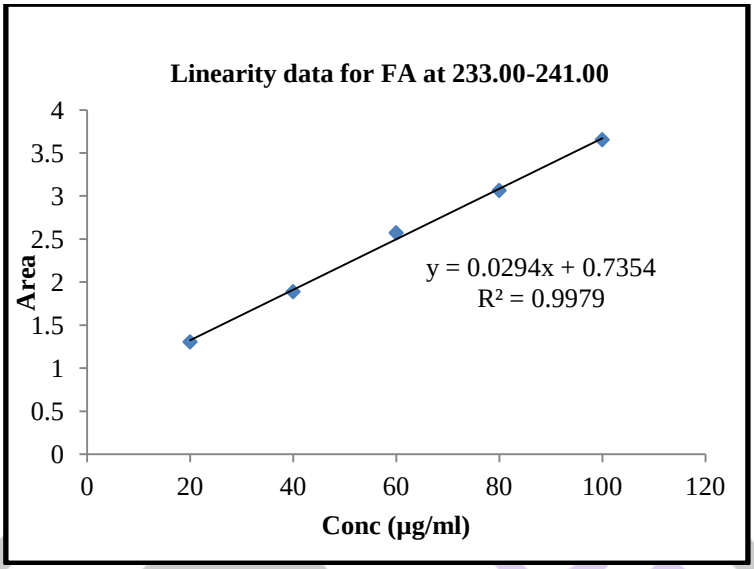

Figure 8: Calibration curve for FA at 233.00 – 241.00 nm

Table 3: Linearity data for MP at 233.00-241.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	2	0.656 ± 0.00296	0.452
2	4	1.230 ± 0.004165	0.437
3	6	1.849 ± 0.006814	0.380
4	8	2.464 ± 0.010183	0.427
5	10	2.988 ±0.0076630	0.257

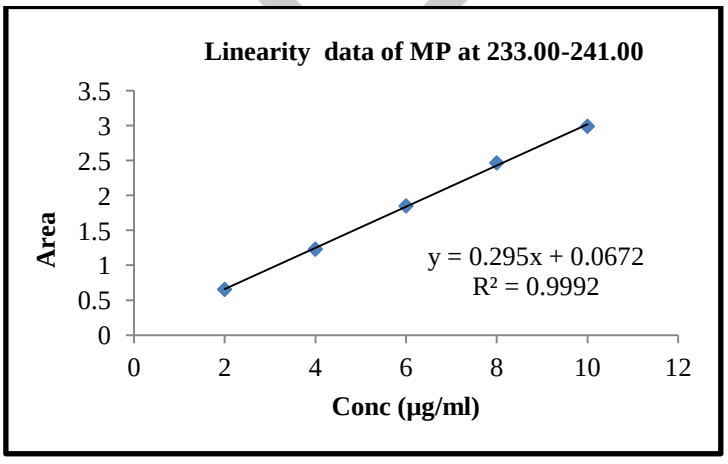

Figure 9: Calibration curve for MP at 233.00 – 241.00 nm

Table 4: Correlation coefficient, regression coefficient and regression line equation for CP,FA & MP

Sr. No.	Drugs	Regression line equation	Regression coefficient (R^2)	Correlation coefficient (r)
1	CP	Y= 0.5666x + 0.5535	0.9968	0.9983
2	FA	Y = 0.0294x + 0.7354	0.9979	0.9989
3	MP	Y = 0.0295x + 0.0672	0.9992	0.9996

Table 5: Linearity data for CP at 220.00 - 228.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	0.5	0.627 ± 0.003191	0.305
2	1	0.836 ± 0.006074	0.641
3	1.5	1.043 ± 0.005149	0.542
4	2	1.293 ± 0.004581	0.466
5	2.5	1.539 ± 0.003494	0.233

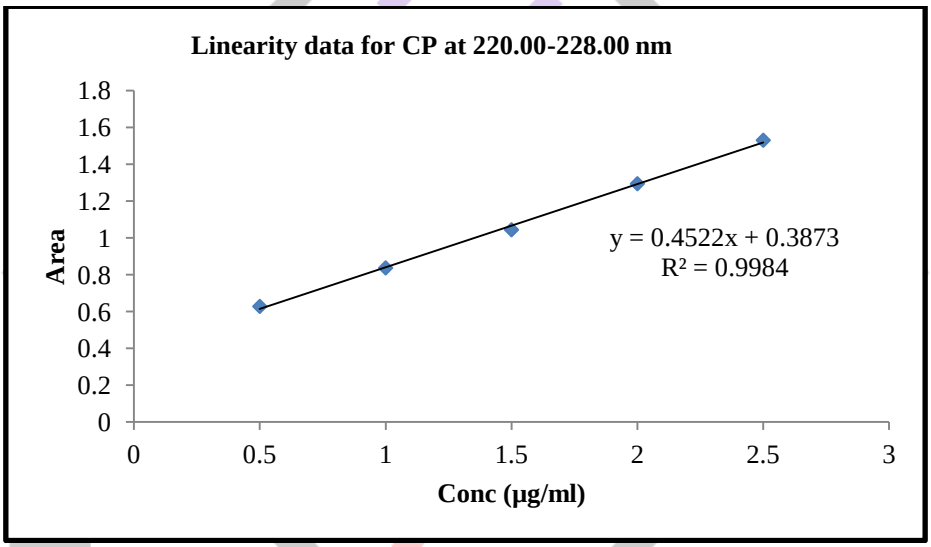

Figure 10: Calibration curve for CP at 220.00- 228.00 nm

Table 6: Linearity data for FA at 220.00-228.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	20	2.664 ± 0.005263	0.197
2	40	3.422 ± 0.005848	0.170
3	60	4.106 ± 0.005069	0.123
4	80	4.647± 0.005372	0.115
5	100	5.454 ± 0.005856	0.107

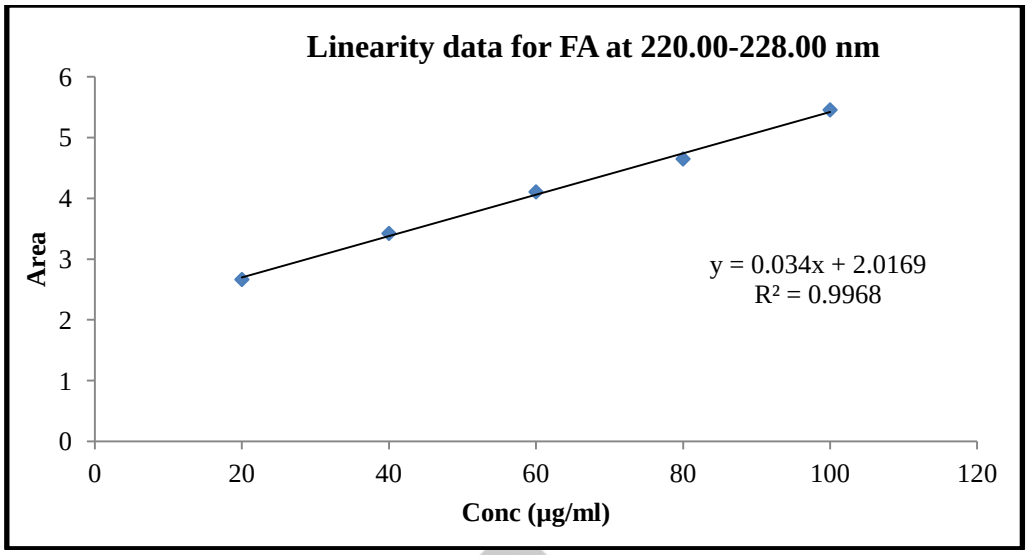

Figure 11: Calibration curve for FA at 220.00- 228.00 nm

Table 7: Linearity data for MP at 220.00-228.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	2	0.137 ± 0.001303	0.959
2	4	0.327 ± 0.002345	0.770
3	6	0.526 ± 0.003039	0.574
4	8	0.709 ± 0.002702	0.381
5	10	0.866 ± 0.002828	0.326

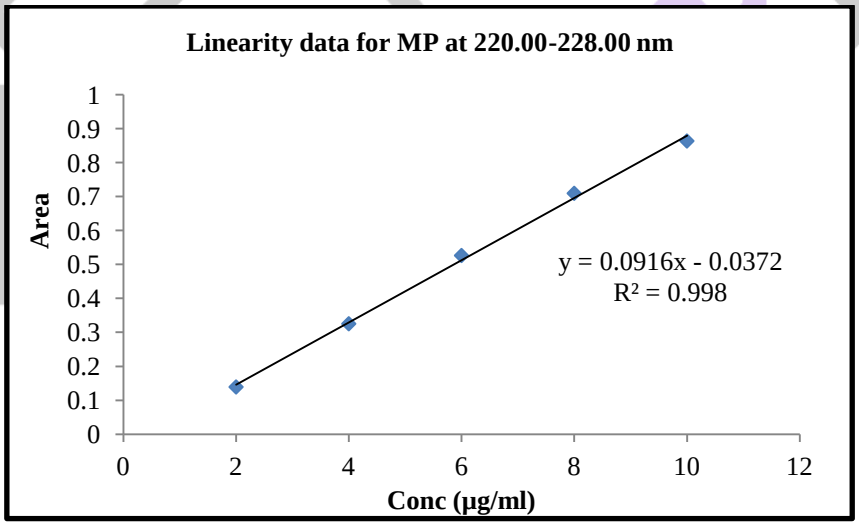

Figure 12: Calibration curve for MP at 220.00- 228.00 nm

Table 8: Correlation coefficient, regression coefficient and regression line equation for CP FA & MP

Sr. No.	Drugs	Regression line equation	Regression coefficient (R^2)	Correlation coefficient (r)
1	CP	Y= 0.4522x + 0.3873	0.9984	0.9992
2	FA	Y = 0.034x + 2.0169	0.9968	0.9983
3	MP	Y= 0.9916x + 0.0372	0.998	0.999

Table 9: Linearity data for CP at 250.00-258.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	% R.S.D.
1	0.5	0.565 ± 0.003263	0.539
2	1	0.723 ± 0.002818	0.405
3	1.5	0.889 ± 0.003059	0.342
4	2	1.158 ± 0.004147	0.371
5	2.5	1.308 ± 0.003116	0.237

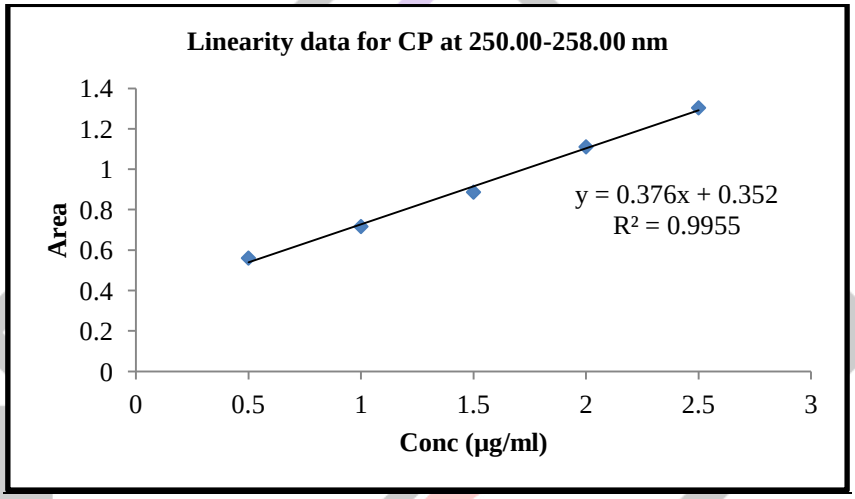

Figure 13 Calibration curve for CP at 250.00- 258.00 nm

Table 10: Linearity data for FA at 250.00-258.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	20	0.054 ± 0.002563	0.528
2	40	0.165 ± 0.002738	0.370
3	60	0.306 ± 0.002069	0.543
4	80	0.448 ± 0.002362	0.445
5	100	0.546 ± 0.002686	0.491

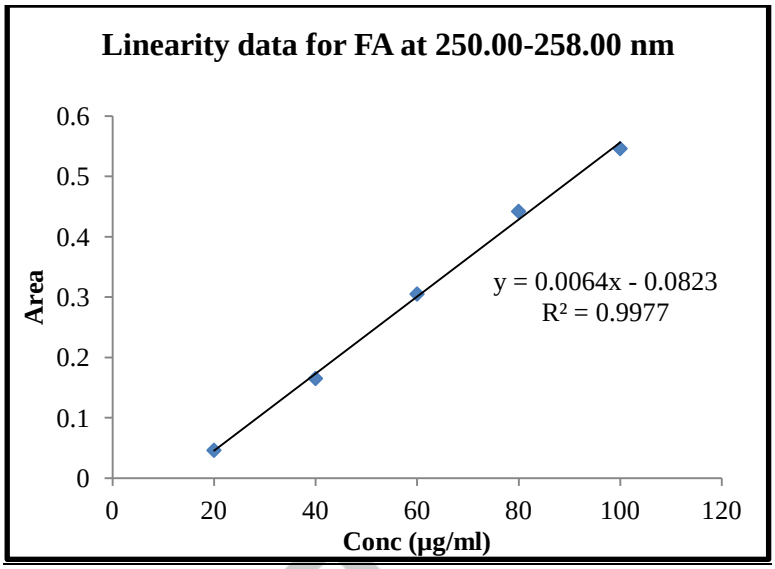

Figure 14: Calibration curve for FA at 250.00- 258.00 nm

Table 11: Linearity data for MP at 250.00-258.00 nm

Sr. No.	Concentration (µg/ml)	Mean AUC ± S.D. (n=5)	%R.S.D.
1	2	1.424 ± 0.010260	0.797
2	4	2.562 ± 0.009848	0.376
3	6	3.896 ± 0.012060	0.313
4	8	5.367 ± 0.041831	0.779
5	10	6.473 ± 0.042678	0.659

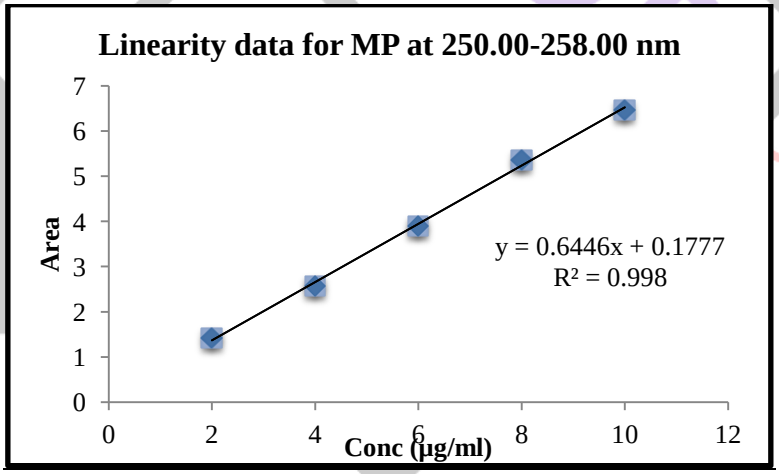

Figure 15: Calibration curve for MP at 250.00- 258.00 nm

Table 12: Correlation coefficient, regression coefficient and regression line equation for CP, FA & MP

Sr. No.	Drugs	Regression line equation	Regression coefficient (R^2)	Correlation coefficient (r)
1	CP	Y= 0.4522x + 0.3873	0.9955	0.9962
2	FA	Y = 0.034x + 2.0169	0.9977	0.9984
3	MP	Y=0.6446x + 0.1777	0.998	0.999

2. Precision

a) Repeatability

The data for repeatability for CP , FA and MP at 233.0- 241.00 nm , 220.00 -228.00 nm and 250.00-258.00 nm

Table 13: Repeatability data of CP , FA and MP

Drugs	Wavelength Range	Concentration (µg/ml)	Mean AUC ± S.D. (n=6)	%R.S.D.
CP	$\lambda_1$233.00-241.00 nm	1	1.117 ± 0.0062	0.354
FA		40	3.444 ± 0.0168	0.497
MP		4	1.230 ± 0.0048	0.391
CP	$\lambda_2$220.00-228.00 nm	1	0.831 ± 0.0052	0.433
FA		40	1.689 ± 0.0073	0.431
MP		4	0.327 ± 0.0021	0.341
CP	$\lambda_3$250.00-258.00 nm	1	0.719 ± 0.0033	0.460
FA		40	0.161 ± 0.0015	0.290
MP		4	2.567 ± 0.0088	0.342

b) Intraday precision

The data for intraday precision for CP, FA and MP at 233.0- 241.00 nm, 220.00 - 228.00 nm and 250.00-258.00 nm

Table 14: Intraday precision data for CP, FA and MP

Drugs	Wavelength Range	Concentration (µg/ml)	Mean AUC ± S.D. (n=6)	%R.S.D.
CP	$\lambda_1$233.00-241.00 nm	1	1.112 ±0.00152	0.131
		1.5	1.364 ± 0.00300	0.214
		2	1.695 ±0.00200	0.115
FA		40	1.675 ± 0.00529	0.314
		60	2.048 ± 0.00378	0.189
		80	2.761 ± 0.00818	0.290
MP		4	1.232 ± 0.00246	0.214
		6	1.847 ± 0.00838	0.453
		8	2.461 ± 0.00814	0.330
CP	$\lambda_2$220.00-228.00 nm	1	0.836 ± 0.00208	0.243
		1.5	1.046 ± 0.00200	0.194
		2	1.294 ± 0.00251	0.196
FA		40	3.428 ± 0.00723	0.214
		60	4.021 ± 0.00757	0.186
		80	4.683 ± 0.01301	0.277
MP		4	0.326 ± 0.00236	0.707
		6	0.526 ± 0.00300	0.570
		8	0.707 ± 0.00152	0.215
CP	λ_3 250.00-228.00 nm	1	0.717 ± 0.00100	0.139
		1.5	0.887 ± 0.00153	0.179
		2	1.130 ± 0.00200	0.199
FA		40	0.163 ± 0.00100	0.613
		60	0.304 ± 0.00234	0.657
		80	0.442 ± 0.00100	0.226
MP		4	2.572 ± 0.00680	0.265
		6	3.889 ± 0.01473	0.378
		8	5.352 ± 0.00577	0.107

c) Interday precision

The data for intraday precision for CP, FA and MP at 233.0- 241.00 nm , 220.00 -228.00 nm and 250.00 – 258.00 nm

Table 15: Interday precision data for CP , FA and MP

Drugs	Wavelength Range	Concentration (µg/ml)	Mean AUC ± S.D. (n=6)	%R.S.D.
CP	$\lambda_1$233.00-241.00 nm	1	1.120 ± 0.01001	0.895
		1.5	1.370 ± 0.00850	0.628
		2	1.708 ± 0.01300	0.766
FA		40	1.681 ± 2.08166	0.529
		60	2.050 ± 2.51500	0.455
		80	2.759 ± 3.07733	0.383
MP		4	1.239 ± 0.00692	0.559
		6	1.844 ± 0.00458	0.248
		8	2.469 ± 0.01350	0.547
CP	$\lambda_2$220.00-228.00 nm	1	0.838 ± 0.00435	0.523
		1.5	1.048 ± 0.00642	0.616
		2	1.297 ± 0.00737	0.569
FA		40	3.439 ± 0.01747	0.505
		60	4.050 ± 0.01307	0.322
		80	4.687± 0.025541	0.547
MP		4	0.326 ± 0.00239	0.707
		6	0.526 ± 0.00300	0.570
		8	0.707 ± 0.00152	0.215
CP	λ_3 250.00-228.00 nm	1	0.759 ± 0.00152	0.212
		1.5	0.889 ± 0.00493	0.547
		2	1.116 ± 0.00611	0.554
FA		40	0.163 ± 0.00152	0.935
		60	0.304 ± 0.00251	0.826
		80	0.444 ± 0.00360	0.821
MP		4	2.579 ± 0.01153	0.448
		6	3.891 ± 0.01644	0.442
		8	5.367 ± 0.01527	0.284

4. Accuracy

Accuracy of the proposed method was assured by performing recovery study from Marketed Formulation at three level by standard addition method. Percentage recovery for CP , FA and MP at 233.00 - 241.00 , 220.00 – 228.00 nm 250.00 – 258.00 nm and was obtained respectively. The results are depicted in Table 16,17 and 18. Recovery was found be in the limit of 98-102 %

Table 16: Determination of Accuracy of CP , FA and MP at 233.00 - 241.00 nm (n=3)

API	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total Amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
CP	0%	1	0	1	0.9971	99.714
	80%	1	0.8	1.8	1.8020	100.116
	100%	1	1	2	2.0114	100.573
	120%	1	1.2	2.2	2.1973	99.877
FA	0%	40	0	40	39.861	99.652
	80%	40	32	72	70.696	99.188
	100%	40	40	80	79.613	100.516
	120%	40	48	88	87.365	99.278
MP	0%	4	0	4	3.9636	98.992
	80%	4	3.2	7.2	7.2068	99.964
	100%	4	4	8	7.9675	100.079
	120%	4	4.8	8.8	8.8076	99.997

Table 17: Determination of Accuracy of CP , FA and MP at 220.00 - 228.00 nm

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total Amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
CP	0%	1	0	1	0.9938	99.381
	80%	1	0.8	1.8	1.7661	98.121
	100%	1	1	2	2.0132	100.661
	120%	1	1.2	2.2	2.2099	100.450
FA	0%	40	0	40	39.943	99.859
	80%	40	32	72	71.983	99.976
	100%	40	40	80	79.410	99.262
	120%	40	48	88	86.810	98.648

MP	0%	4	0	4	3.9537	98.992
	80%	4	3.2	7.2	7.1768	98.990
	100%	4	4	8	7.9252	100.079
	120%	4	4.8	8.8	8.8026	99.987

Table 18: Determination of Accuracy of CP , FA and MP at 250.00 - 258.00 nm

Drugs	Level	Amount of sample (µg/ml)	Amount of std. spiked (µg/ml)	Total Amount (µg/ml)	Amount of sample found (µg/ml)	% Recovery
CP	0%	1	0	1	0.9358	99.480
	80%	1	0.8	1.8	1.7923	98.423
	100%	1	1	2	2.0423	100.717
	120%	1	1.2	2.2	2.2054	100.660
FA	0%	40	0	40	39.978	99.768
	80%	40	32	72	71.956	99.879
	100%	40	40	80	79.564	99.345
	120%	40	48	88	86.956	98.557
MP	0%	4	0	4	3.9434	98.590
	80%	4	3.2	7.2	7.2678	100.940
	100%	4	4	8	7.9950	100.089
	120%	4	4.8	8.8	8.7706	99.665

- Analysis of Marketed formulation (sample- Cream): Suitability of the method was tested by analyzing the Marketed Formulation. The results are depicted in Table. 19

Table 19: Determination of Assay of CP , FA and MP (LOZIVATE ®–F CREAM)

Cream	Actual Concentration (µg/ml)		Amount obtained Mean ±S.D. (µg/ml) (n=5)		% Drug recovery ± S.D (n=5)	
	CP	1	CP	0.9934 ± 0.00115	% CP±S.D.	98.416 ± 0.0289

	FA	40	FA	39.860 ± 0.0467	% FA±S.D	99.650 ± 0.3046
	MP	4	MP	3.9480 ± 0.00465	% MP±S.D	99.756 ± 0.0307

- SUMMARY OF VALIDATION PARAMETER FOR PROPOSED METHOD

Table 20: Summary of Area Under Curve Method

Parameters	CP	FA	MP
Wavelength (nm)	233.00-241.00 nm	220.00-228.00 nm	250.00-258.00 nm
Linearity (µg/ml) (n=5)	0.5-2.5 µg/ml	20-100 µg/ml	2-10 µg/ml
Regression Equation (y = mx + c)	Y= 0.5666x + 0.5535	Y = 0.034x + 2.0169	Y = 0.0295x + 0.0672
Regression coefficient (R^2)	0.9968	0.9968	0.9992
Correlation Coefficient (r)	0.9983	0.9983	0.9996
Repeatability (%R.S.D.) (n=6)	0.5566	0.4376	0.3424
Intraday precision (%R.S.D) (n=3)	0.1179 - 0.1370	0.2110-0.2786	0.2142 - 0.3331
Interday precision (%R.S.D.) (n=3)	0.7612- 0.8934	0.5080-0.5448	0.2153 - 0.7073
LOD (µg/ml) (n=5)	0.0547	0.6069	0.3005
LOQ (µg/ml) (n=5)	0.1660	1.8391	0.9092
% Recovery (n=3)	99.71- 100.57	98.648-99.859	98.99 – 100.07
Assay (%) ± S.D. (n=5)	98.416 ±0.02891	99.650 ±0.3046	99.756 ±0.0307

5 Conclusion

Based on the results, obtained from the analysis of CP,FA and MP in their Marketed Formulation using Area Under Curve Method, It can be concluded that the linearity range was found to be 0.5- 2.5 µg/ml for Clobetasol propionate and 20-100 µg/ml for Fusidic acid and 2-10 µg/ml for Methyl Paraben. The co-relation co-efficient was found to be 0.9983, 0.9989 and 0.9992 for CP , FA and MP at λ_1 233.00 -241.00 nm , λ_2 220.00 – 228.00 nm and λ_3 250.00 – 258.00 nm respectively. The % recovery were found to be 99.71- 100.57 , 99.38- 100.45 and 99.65- 100.51 for CP, FA and MP at λ_1 233.00 -241.00 nm , λ_2 220.00 – 228.00 nm and λ_3 250.00 – 258.00 nm respectively. Intraday precision were found to be 0.11-0.13,0.29-0.31, 0.21 - 0.33 % RSD for CP, FA and MP at λ_1 233.00 -241.00 nm , λ_2 220.00 – 228.00 nm and λ_3 250.00 – 258.00 nm respectively. Interday precision were found to be within the range.

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