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Development and Validation of Stability Indicating Implementation of Quality by Design (QbD) Approach RP-HPLC Method for Quantitative Estimation of Metaxalone in Tablet Dosage Form

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ABSTRACT

A new, simple, rapid, selective, precise, and accurate isocratic reverse-phase high-performance liquid Chromatography assay method has been developed to estimate Metaxalone in tablet formulations. The separation was achieved using column Zorbax SB-AQ (150x4.6mm, 3.5µ) in the mobile phase consisting of pH 2.5 phosphate buffer and Acetonitrile. The flow rate was 1.5 mL/min, column oven temperature and sampler cooler was maintained 25°C, respectively. The Metaxalone was detected using a U.V. detector at the wavelength of 220 nm, and the injection volume was 10µL. Metaxalone's retention time was noted to be 4.40 min, respectively, indicative of rather shorter analysis time. The method was validated as per ICH guidelines. The proposed method was found to be accurate, reproducible, and consistent.

Keywords: *Metaxalone, Liquid chromatography, Force Degradation, QbD, and Validation.*

I. INTRODUCTION

Metaxalone, chemically 2-[(3, 4-dimethyl phenoxy) methyl]-2-oxazolidinone is a centrally acting muscle relaxant [1]. Metaxalone's mechanism of action in humans has not been established but may be due to general central nervous system depression [2]. Metaxalone has no direct action on the contractile mechanism of striated muscle, the motor endplate, or the nerve fiber. There is very limited or inconsistent data regarding the effectiveness and safety of Metaxalone [3]. Metaxalone is a commonly used drug in muscle relaxant therapies for acute lower back pain [4]. Analytical methods using RP-HPLC [5-6], liquid chromatography-mass spectrometry (LC-MS/MS) [7-9], and ultra-violet (U.V.) spectroscopy [10-11] have been successfully reported for Metaxalone quantification. However, none of them adopt systematic statistical optimization; rather, those methods pursued the traditional approach, i.e., varying one factor at a time while keeping the others constant. Since the RP-HPLC method optimization is a complex process, various parameters and chemical factors such as mobile phase pH, buffer concentration, flow rate, column temperature, etc. are to be simultaneously monitored to achieve separation selectivity and other performance criteria [12]. Moreover, the traditional approach based on trial and error methodologies is inefficient and time consuming and may not identify the optimal condition [13-16]. QbD study also performed, and critical parameters need to be controlled to fulfill ATP (analytical target profile) requirements are identified.

The present study illustrates the development and validation of a simple, accurate, and precise procedure for Metaxalone's determination by RP-HPLC.

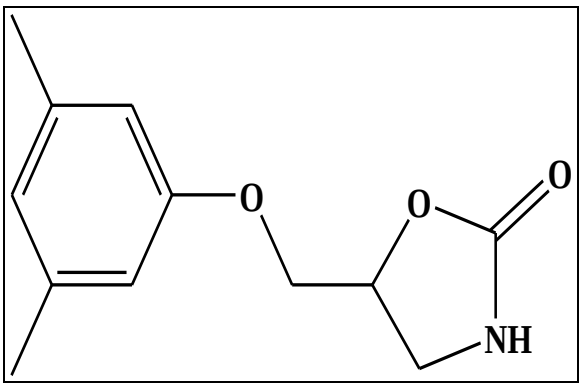

Figure:1 Structure of Metaxalone

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II. EXPERIMENTAL

Chemicals and reagents:

Potassium dihydrogen orthophosphate, Orthophosphoric acid, Acetonitrile, and water was from Merck chemicals Mumbai, India. Mumbai, India. 0.45µm PVDF filter and 0.45µ Nylon membrane filter were from Millex-HN, Millipore Mumbai, India.

Instrumentation:

Agilent 1200 series with open lab software, Bandelin ultrasonic bath, pH Meter (Thermo Orion Model), Analytical Balance (Sartorius), and Microbalance (Mettler Toledo Model) were used in the present Assay.

Preparation of pH 2.5 Phosphate Buffer (10mM):

1.36gms of potassium dihydrogen orthophosphate was accurately weighed and transferred in 1000ml beaker, added 1000ml of HPLC grade water sonicated to dissolve. The solution's pH was adjusted to 2.5 with orthophosphoric acid and made up to volume with water. The answer was filtered through a 0.45µm membrane filter.

Preparation of mobile phase-A:

She mixed accurately 950 mL buffer and 50mL acetonitrile in the (95:5 v/v) ratio.

Preparation of mobile phase-B:

She mixed accurately 250 mL buffer and 750mL acetonitrile in the ratio of (25:75 v/v).

Diluent preparation:

I mixed accurately 500 mL buffer and 500mL acetonitrile in the (50:50 v/v) ratio.

Standard stock preparation: Accurately weighed 50.0 mg of Metaxalone working standard was transferred into a 50 ml volumetric flask. 25 ml of diluent was added and sonicated to dissolve. The solution was diluted to volume with diluent and mixed well.

Standard preparation:

Transferred 5ml of the standard stock solution was into 100ml volumetric flask and volume made up to the mark with diluent (50 ppm).

Sample preparation: An accurately weighed portion of a powder equivalent to about 500 mg of Metaxalone was transferred to a 500 ml volumetric flask. 300 ml of diluent was added and sonicated to dissolve for 20 minutes with intermediate shaking. Cool to room temperature, dilute up to volume with diluent, and mixed well and centrifuged the above sample solution at 5000rpm for 5minutes. 5ml of supernatant from the above solution was pipetted into 100ml volumetric flask and volume made up to the mark with the diluent filtered the solution through 0.45µm PVDF syringe filter (50 ppm).

Chromatographic conditions: Chromatographic analysis was performed on Zorbax SB-AQ 150x4.6mm, 3.5µ column. The mobile phase-A consisted of pH 2.5 phosphate buffer and Acetonitrile (95:5 v/v). The mobile phase-B consisted of pH 2.5 phosphate buffer and Acetonitrile in the ratio of (25:75 v/v). The flow rate was 1.5mL/min, column oven temperature, and sample cooler 25°C, the injection volume was 10µL, and detection was performed at 220 nm using a photodiode array detector (P.D.A.).

III. RESULTS & DISCUSSION

Method development: Spectroscopic analysis of compound Metaxalone showed maximum U.V. absorbance (λmax) at 220 nm. To develop a suitable and robust L.C. method for Metaxalone's determination, different mobile phases were employed to achieve the best separation and resolution.

Trial: 1

Chromatographic conditions:

Mobile phase : Water: ACN: Methanol (450:100:450)
Flow rate : 1.5 ml/min
Colum : Inertsil ODS 4 (4.6100) mm, 5µ
Detector wavelength : 220nm
Column temp : 30ºC
Injection volume : 20µl
Run time : 10mins

In Trial-1, peak shape and Metaxalone were found good, but impurity3 peaks were merged with the main peak. Hence method needs to improve further to increase the resolution between the main peak and impurities.

Trial: 2

Chromatographic conditions:

Mobile phase: Buffer : water: methanol (30:70)
Flow rate : 1ml/min
Column : Symmetry C18 (150x 4.6) mm, 5m
Column temp : 50ºC
Injection volume : 20µl
Run time : 30mins

Metaxalone's peak shape was found not good, and impurity peaks were merged with the main peak; hence, the method further needs to improve the peak shape and resolution.

Trial: 3

Chromatographic conditions:

Mobile phase A : Buffer (pH 4.5) 10mM
Mobile phase B : Acetonitrile
Column : Zorbax SB-AQ (150x 4.6) mm, 3.5m
Column temp : 25ºC
Injection volume : 10µl

After conducting the above trials, it was found that the isocratic mode is not useful, so moved to the gradient mode of separation to get good separation. The particle size of the column decreased to 3.5µ from 5µ to get good separation. To prevent the broadening of peak shape, injection volume was decreased to 10µL from 20 µL because higher injection volume leads to fronting.

Metaxalone's peak shape was found good, but impurity-2 and impurity-C peaks were co-eluted with the main peak. Hence gradient mode needs to modify further to get a good resolution.

Trial: 4

Chromatographic conditions:

Mobile phase A : Buffer (pH2.5) 10mM:Acetonitrile(95:50)
Mobile phase B : Buffer (pH 2.5) 10mM:Acetonitrile(25:75)
Flow rate : 1.5ml/min
Run time : 10min

Gradient Elution Optimization

Time in Minutes	Flow	%A	%B
0.01	1.5	60	40
4.0	1.5	45	55
6.0	1.5	00	100
7.0	1.5	60	40
10.0	1.5	60	40

Metaxalone's peak shape was found good, and all impurity peaks were well separated from the main peak. Hence this method was finalized to conduct further studies and validation parameters according to ICH guidelines.

IV. METHOD VALIDATION

The developed RP-HPLC method extensively validated for Assay of Metaxalone using the following parameters.

A. Specificity:

Preparation of blank solution:

The diluent is used as a blank solution.

Preparation of Placebo solution: An accurately weighed portion of a placebo, equivalent to about 500 mg of Metaxalone, is transferred to a 500 ml volumetric flask. 300 ml of diluent was added and sonicated to dissolve for 20 minutes with intermediate shaking. Cool to room temperature,

We centrifuged the above solution at 5000rpm for 5min. 5ml of supernatant from the above solution pipetted into 100ml volumetric flask volume made up to the mark with diluent filtered the solution through a 0.45µm PVDF syringe filter.

The Chromatogram of Metaxalone standard using the proposed method is shown in **(Figure:2)** system suitability results of the method are presented in **Table:1.**

Blank and Placebo interference: A study to establish the interference of blank and placebo were conducted. Diluent and placebo were injected into the chromatograph in the defined above chromatographic conditions, and the blank and placebo chromatograms were recorded. **Figure: 3 & 4.**

Table: 1 Impurity interference data

Peak Name	Retention Time	Blank	Placebo
Metaxalone	4.65	No interference	No interference

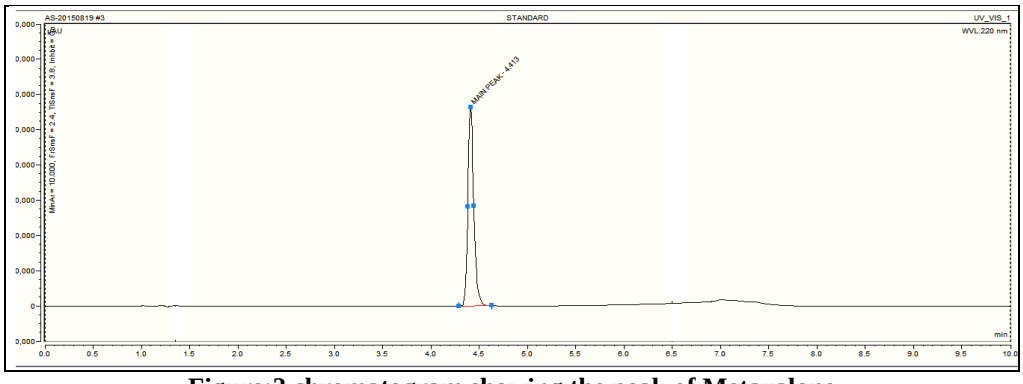

Figure:2 chromatogram showing the peak of Metaxalone

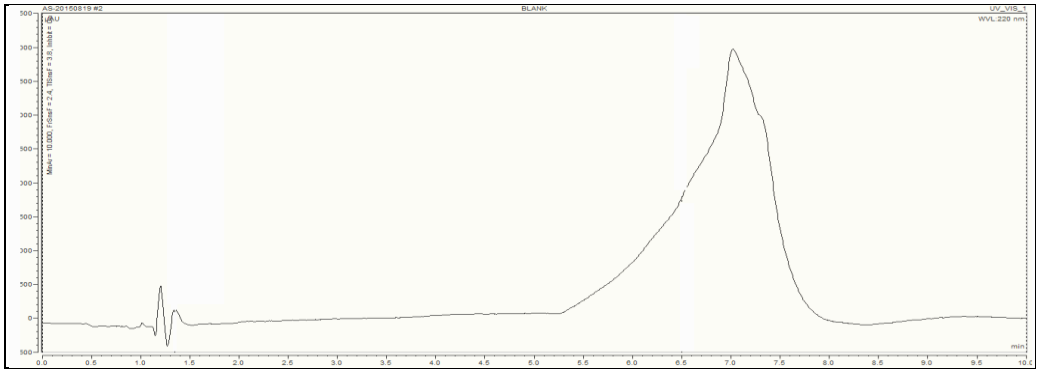

Figure:3 chromatogram showing the no interference of Diluent for Metaxalone.

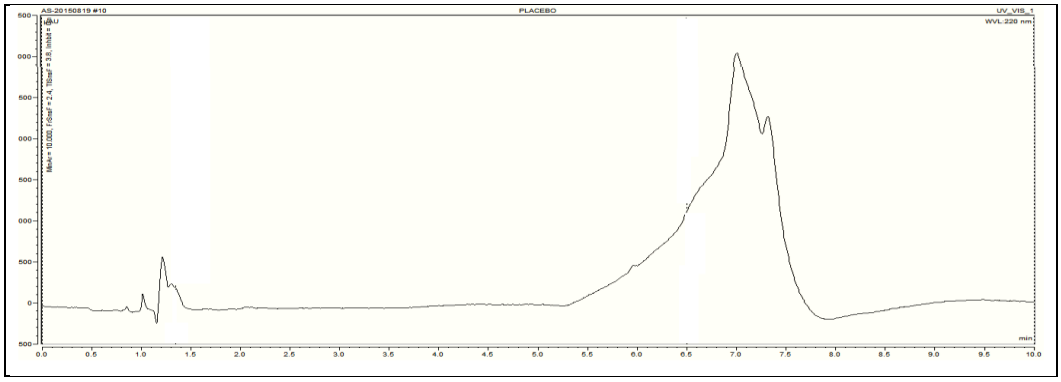

Figure: 4 Chromatogram showing no interference of placebo for Metaxalone.

- The chromatogram of the blank solution showed no peak at the retention time of the Metaxalone peak. This indicates that the diluent solution used in sample preparation does not interfere in estimating Metaxalone in Metaxalone tablets.
- Similarly, the placebo solution's chromatogram showed no peaks at the retention time of the Metaxalone peak. This indicates that the placebo used in sample preparation does not interfere in estimating Metaxalone in Metaxalone tablets.

Force Degradation studies:

Table: 2 Forced degradation results of Metaxalone

Stress Type	Degradation condition	%Assay	% of Degradation
As such	Controlled sample	100.8	NA
Oxidative Degradation	30% H_2O_2solution and heat it at 80ºC for 3 hours	98.5	2.3
Acid degradation	5.0 N HCl and heat it at 80ºC for 3 hours.	99.5	1.3
Alkali (Base degradation) degradation	5.0 N NaOH and heat it at 80ºC for 3 hours.	40.7	60.1
Thermal Degradation	60ºC in the oven for 7 days.	99.4	1.4
Humidity degradation	40ºC and 75% R.H. for 7 days.	99.2	1.6
Photolytic Degradation	Photolytic Degradation Exposure to 1.2 million lux hours at 200-watt hours/square meter ultraviolet energy	98.3	2.5

- Significant Degradation was observed in the alkali stress condition. Hence it can be concluded that Metaxalone Tablets is sensitive to alkali.

B. Precision

a) System Precision:

Perform the study of standard six times and determine the %RSD of peak area of replicate injections of Metaxalone

.

Table: 3 System Precision data for Metaxalone

Injection No	Metaxalone
1	484255
2	484258
3	484532
4	483921
5	484318
6	483904
Mean area	**484198**
SD	**243.298171**
%RSD	**0.05**

The %RSD of peak area for Metaxalone was found to be 0.05% below 2.0%, indicating that the system gives precise results.

b) Method precision: The test method's precision was evaluated by doing an Assay for six Metaxalone tablet samples as per the test method. The content in mg and % label claim for Metaxalone for each of the test preparation was calculated. The average content of the six preparations and % R.S.D. for the six observations were calculated. The chromatogram was shown in **Figure:5,** and data were shown in **Table:4.**

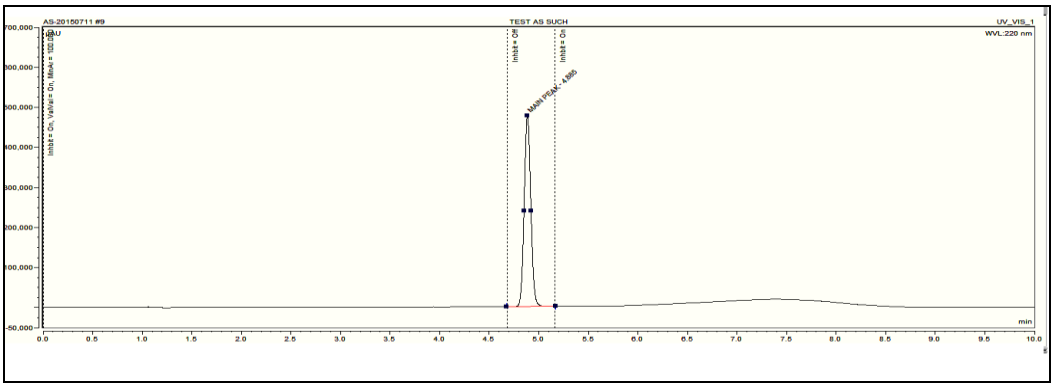

Figure:5 Method precision sample chromatogram

Table:4 Method precision data for Metaxalone

No. of injections	**Metaxalone**
	% assay
1	100.9
2	99.3
3	100.4
4	99.5
5	100.5
6	100.3
Average	**100.2**
%RSD	**0.62**

c) Intermediate precision:

Intermediate precision of the test method was demonstrated by preparing six preparations of the same sample. Representing a single batch by two different analysts on different days, the same column but different lot numbers, different HPLC systems, and these samples were prepared as per the test method. The intermediate precision was evaluated by calculating % relative standard deviation. And % overall relative standard deviation of the impurities.

Table: 5 Intermediate precision data for Metaxalone

No. of injections	Metaxalone
	% assay
1	99.8
2	99.5
3	100.2
4	100.6
5	100.3
6	99.4
Average	99.97
%RSD	0.48

Table: 6 Comparison between Method precision and Intermediate precision

No. of Injections	Metaxalone	
	% Assay	
	MP	IP
1	100.9	99.8
2	99.3	99.5
3	100.4	100.2
4	99.5	100.6
5	100.5	100.3
6	100.3	99.4
Overall Avg.	100.06	
Overall STD	0.54	
Overall % RSD	0.53	

- For six sample solutions (method precision and intermediate precision), the % of Assay found within the specification limit.
- For six sample solutions (method precision and intermediate precision), the % relative standard deviation for Metaxalone was meeting the acceptance criteria.
- For twelve sample solutions (method precision and intermediate precision), the overall % relative standard deviation for Metaxalone was meeting the acceptance criteria.

C. Linearity of detector response:

The standard curve was obtained in the concentration range of 12-76 µg/ml for Metaxalone. The linearity of this method was evaluated by linear regression analysis. Slope, intercept, and correlation coefficient [r2] of standard curves were calculated and given in Figure:5 to demonstrate the proposed method's linearity. From the data obtained, which is given in Table:7 the method was linear within the proposed range.

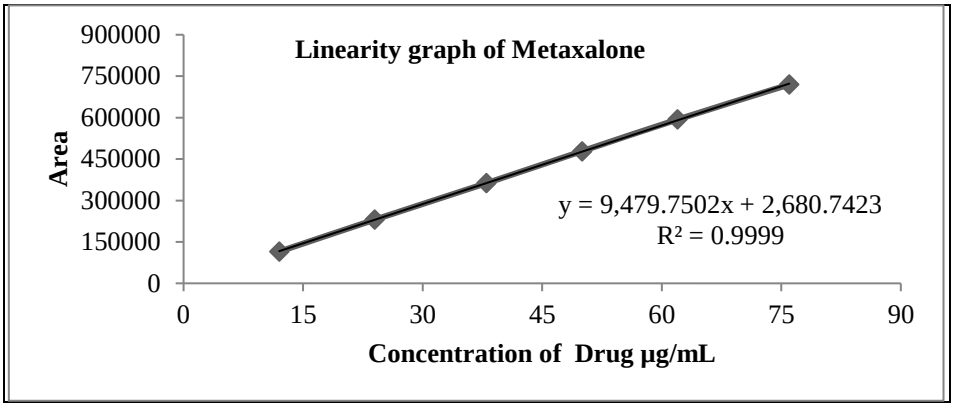

Figure:5 Calibration curve for Metaxalone

Table: 7 Linearity studies for Metaxalone by the proposed method

S.No	Linearity Level (%)	Concentration (µg/ml)	Area
1	25	12	114836
2	50	24	230672
3	75	38	362647
4	100	50	478484
5	125	62	593320
6	150	76	719820
Slope			9479.7502
Intercept			2680.7423
r^2			0.9999
% Y-Intercept			0.56

- The Coefficient of determination for Metaxalone peak was meeting the acceptance criteria.
- The % Y-intercept for Metaxalone peak was meeting the acceptance criteria.
- The linearity was established, and results are found satisfactory.

D. Accuracy:

The accuracy of the method was determined on three concentration levels by recovery experiments. The recovery studies were carried out in triplicate preparations on composite blend collected from 20 tablets of Metaxalone, analyzed as per the proposed method. The percentage recoveries with found in the range of 100.04 to 100.47 for Metaxalone. The data obtained, which is given in **Table:8** the method was found to be accurate.

Table:8 Recovery studies for Metaxalone by the proposed method

Recovery Level	Amount Added (mg)	Amount recovered (mg)	% Recovery	% Mean Accuracy
50%	250.36	250.24	99.97	100.47
	251.02	253.37	100.96	
	250.78	251.94	100.48	
100%	500.80	502.53	100.37	100.3
	501.00	504.67	100.75	
	500.70	499.49	99.78	
150%	750.10	750.75	100.09	100.04
	750.15	751.82	100.22	
	750.90	749.45	99.81	

E. Chromatographic DoE

The design of the experiment should be made for chromatographic conditions and sample matrix separately. Several runs to be conducted for chromatographic conditions in the following table are based on the DoE.

Table: 9 Results of Chromatographic DoE

Std	Run	Critical Quality Method Attributes		
		Retention Time	Tailing Factor	Theoretical plates
16	1	3.767	1.15	22107
10	2	3.947	1.24	23644
8	3	3.753	1.1	22767
1	4	5.320	1.34	35674
14	5	3.600	1.28	21813
2	6	3.907	1.27	25084
15	7	4.387	1.09	25139
9	8	4.620	1.22	29279
12	9	3.487	1.09	19581
18	10	4.340	1.27	27249
13	11	5.007	1.29	30503
6	12	4.380	1.29	27318
4	13	4.120	1.08	23575
19	14	4.360	1.13	25061
3	15	4.707	1.12	24343
17	16	4.393	1.08	25216
11	17	4.747	1.03	23508
7	18	5.140	1.03	27790
5	19	4.940	1.22	31501

Std: Standard order

After conducting all the chromatographic runs based on the results obtained, plotted pareto charts to identify which parameter shows more effect on each critical quality attribute and know the parameters to be critically controlled (essential parameters of method) to get the desired essential attributes of quality.

a) Retention Time of API Peak:

Pareto chart

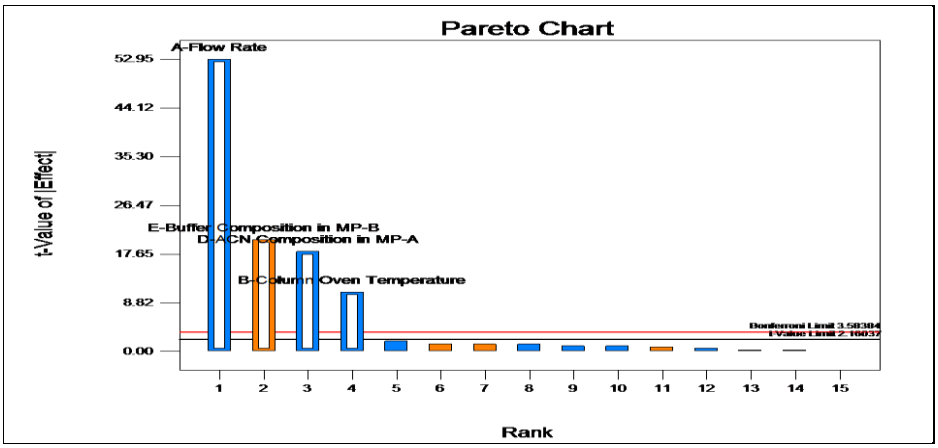

Figure:6 Retention Time of API Peak

After observing all the factors effects on the retention time of Metaxalone peak by using the above chart it was concluded that the flow rate parameter was shown to affect the retention time of the Metaxalone peak.

b) Tailing Factor of API Peak:

Pareto Chart:

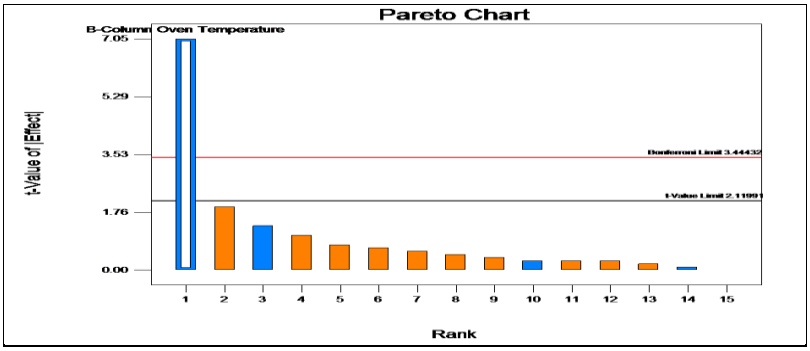

Figure:7 Tailing Factor of API Peak

After observing all the factors effects on tailing factor of Metaxalone peak by using the above chart, it was concluded that the column oven temperature parameter was shown more effect on tailing factor of Metaxalone peak

c) Theoretical plates of API Peak:

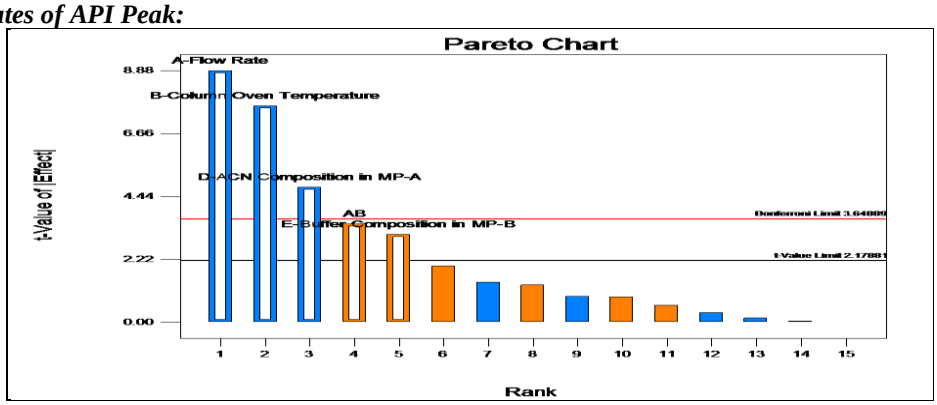

Figure:8 Theoretical plates of API Peak

After observing all the factors' effects on the Metaxalone peak's theoretical plates by using the above chart, it was concluded that the flow rate parameter was shown more effect on the theoretical plates of the Metaxalone peak.

By using all the results obtained for chromatographic DoE runs a range of different method parameters decided, in that range, only this method gives desired critical quality attributes.

Table:10 Method Operable Design Range

S.No.	Critical Quality Method Parameters	MODR	Set Level
1	Column oven temperature (°C)	20	25
2	Flow Rate (mL/min)	1.4	1.6
3	a pH of Buffer solution	2.30	2.50
4	Composition of Acetonitrile in Mobile Phase-A (mL)	35	50
5	Composition of Buffer in Mobile Phase-B (mL)	230	250

Key observations:

- **Retention Time of API**: The value of the retention time of API decreases with increased Flow rate levels, Acetonitrile composition in mobile phase-A and Column oven temperature, and vice versa. The value of the Retention time of API increase with an increase in the level of Buffer composition in Mobile phase-B and vice versa. The change in the buffer solution's pH did not significantly affect the value of API's retention time.
- **Tailing Factor of API:** The value of Tailing factor API decreases with an increase in Column oven temperature and vice versa. The remaining factors Flow rate, pH of buffer solution, Acetonitrile composition in Mobile phase-A, and buffer composition in Mobile phase-B did not significantly affect the Tailing factor of API.
- **Theoretical plates of API:** The value of Theoretical plates of API decrease with the increase in the levels of Flow rate, Column Oven Temperature, and Acetonitrile composition in the Mobile phase and vice versa. The value of Theoretical plate counts increases with an increase in the level of Buffer composition in Mobile phase-B and vice versa.

F. Sample matrix DoE

Details of preparations and procedures for respective sample matrix DoE run results of sample matrix DoE runs were shown in the following table

.

Table: 11 Results of Sample Matrix DoE

Std	Block	Run	pH of buffer	Acetonitrile Composition in diluent (mL)	Sample Quantity (mg)	Volume of diluent (mL)	Sonication Time (min.)	% Assay
9	Block 1	1	2.00	400	250	350	10	99.9
6	Block 1	2	3.00	400	750	250	30	100.8
8	Block 1	3	3.00	600	750	250	10	102.2
15	Block 1	4	2.00	600	750	350	10	100.7
14	Block 1	5	3.00	400	750	350	10	100.7
3	Block 1	6	2.00	600	250	250	10	100.9
2	Block 1	7	3.00	400	250	250	10	101.2
5	Block 1	8	2.00	400	750	250	10	99.2
4	Block 1	9	3.00	600	250	250	30	101.5
11	Block 1	10	2.00	600	250	350	30	100.1
7	Block 1	11	2.00	600	750	250	30	100.9
10	Block 1	12	3.00	400	250	350	30	100.6
12	Block 1	13	3.00	600	250	350	10	101.2
17	Block 1	14	2.50	500	500	300	20	101.5
13	Block 1	15	2.00	400	750	350	30	99.1
1	Block 1	16	2.00	400	250	250	30	99.9
18	Block 1	17	2.50	500	500	300	20	102.5
16	Block 1	18	3.00	600	750	350	30	102.7
19	Block 1	19	2.50	500	500	300	20	101.6
20	Block 2	20	2.50	500	500	250	20	101.1
21	Block 2	21	2.50	500	250	300	20	101.3
22	Block 2	22	2.50	600	500	300	20	101.2
23	Block 2	23	3.00	500	500	300	20	101.9
24	Block 2	24	2.50	500	500	300	10	101.0

After conducting all the sample matrix, DoE runs based on the results obtained plotted pareto charts to identify which parameter shows more effect on %recovery and know the parameters to be critically controlled (critical method parameters) to get the desired %recovery.

G. %Recovery (% Assay of Metaxalone)

Pareto Chart:

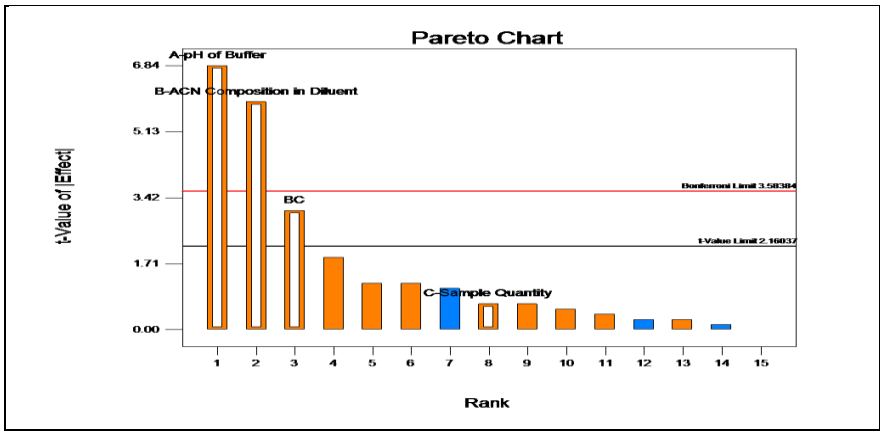

Figure: 9 Recovery (% Assay of Metaxalone)

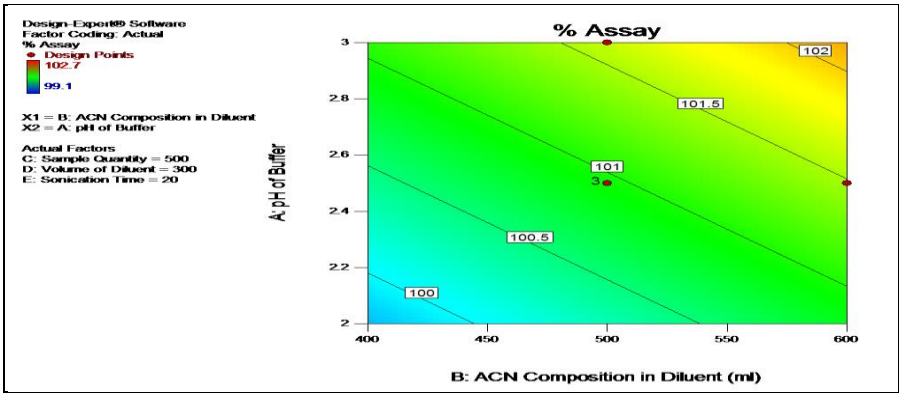

Figure: 10 Recovery (% Assay of Metaxalone)

After observing all the results, it was concluded that the value of % Assay of Metaxalone increases with increased pH levels of the buffer used in diluent and composition of Acetonitrile in Diluent and vice versa.

Using all the results obtained for the sample matrix, DoE runs a range of different method parameters. In that range, only this method gives the desired %recovery.

Table: 12 Method Operable Design Range:

S.No.	Critical Quality Method Parameters	MODR	Set Level
1	the pH of buffer used in Diluent	2.00 – 3.00	2.50
2	Acetonitrile composition in Diluent (mL)	400 – 600	500
3	Sample Quantity (mg)	250 – 750	500
4	Volume of Diluent added in sample preparation (mL)	250 - 350	300
5	Sonication Time (Minutes)	10 - 30	20

Key observations:

❖ **% Assay of Metaxalone:**

The value of % Assay of skeletal muscle relaxant drug decrease with decrease in levels of pH of buffer used in Diluent and Acetonitrile composition in diluent and vice versa, but % Assay of skeletal muscle relaxant drug is within the limit (98.0 to 102.0%) by changing the level of pH of the buffer from 2.00 to 3.00 and Acetonitrile composition in diluent from 400 to 600ml.

V. CONCLUSION

An RP-HPLC method for the estimation of Metaxalone was developed and validated as per ICH guidelines. The results obtained indicate that the proposed method is rapid, accurate, selective, and reproducible. Linearity was observed over a concentration range of 12-76µg/ml. The regression coefficient for the linearity of the method was found as 0.9999. The accuracy of the method was found between 100.04 to 100.47 in all three levels. %RSD for both method precision and intermediate precision, was found as 0.48 and 0.62, respectively. Forced degradation data revealed the drug is stable in all the degradation conditions except in alkali. After conducting

As there is no interference of blank and placebo at the retention time of Metaxalone. It is very fast, with good reproducibility and good response. Validation of this method was accomplished, getting results meeting all requirements. The method is simple, reproducible, with good accuracy and linearity. It allows the analysis of Metaxalone in its different pharmaceutical dosage forms reliably.

QbD study critical process parameters need to be controlled were identified. Those are for the retention time with flow rate, for the tailing factor with column oven temperature, for the theoretical plates.

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REFERENCES

[1] K. B. Gabhane, V. Kasture, N. Shrikhande, N. Barde, and P. Wankhade, (2009) "*Simultaneous Spectrophotometric Determination of Metaxalone and Diclofenac Potassium in Combined Tablet Dosage form*", Int. J. Chem. Sci., 7(1), 539-545.

[2] A. C. Gruszecki, S. Kloda, G. T. Simmons, T. M. Daly, R. W. Hardy, and C. A. Robinson, (2003) "*Polydrug Fatality Involving Metaxalone*", J. Forensic Sci., 48, 432-434.

[3] D. Nissen, Ed. Mosby's Drug Consult & Trade, (2003)13th Ed. Mosby, Inc., St. Louis, MO.

[4] Physicians' Desk Reference, 58th Ed. Medical Economics Co., Montvale, NJ 2004; p. 2181.

[5] Sahu P.K., Annapurna M, Saho D.K. (2011) "*Development and validation of stability indicating RP-HPLC method for determining Metaxalone in bulk and its pharmaceutical formulations*". E J Chem 8: S439-S447.

[6] Krishna MV, Dash RN, Saritha V, Shravani D, Ramesh D, Madhavi, G. (2012) "*Kinetics study of Metaxalone degradation under hydrolytic, oxidative, and thermal stress conditions using stability-indicating HPLC method*". J Pharm Anal 2: 431- 436.

[7] Nageswara RN, Farah H, Sahu PK, Janarthan M, Naidu G, ChG. (2013) "*Development and validation of a stability-indicating Assay, including the isolation and characterization of Metaxalone's degradation products by LC-MS*". Biomed Chromatogr 27(12): 1733-40.

[8] Ramakrishna N, Kandikere VS, Viswottam N, Shukla M, Koteshwara M, Wishu S, Praveen, V.D. (2006) "*Quantification of Metaxalone in human plasma by liquid chromatography coupled to tandem mass spectrometry"*. J Anal Toxicology 30: 245-251.

[9] Goswami D, Saha A, Gurule S, Khuroo A, Monif T, Poonam V. (2012) "*Metaxalone estimation in a biological matrix using high throughput L.C.–MS/MS bioanalytical method*". J Chrom B, 902: 132-136.

[10] Priyadharsini J, Gigi GP, Niraimathi V, Suresh AJ. (2010) "*The area under the curve and second-order derivative spectroscopy of Metaxalone in bulk drug and tablet formulation"*. Int J Chem Sci, 8: 823-827.

[11] Rao RN, Anil K.B., Laxmi RA, Vamshi RA, Sreenivasulu K. (2011) "*Method validation of Metaxalone drug by using U.V. spectroscopy*". Int J Pharm Bio Sci 1: 284-302.

[12] Vander HY, Nijhuis A, Smeyers-Verbeke J, Vandeginste B.G.M., Massart DL. (2001) "*Guidance for robustness: ruggedness tests in method validation*." J Pharm Biomed Anal 24: 723-753.

[13] Singh B, Kumar R, Ahuja N. (2005) "*Optimizing drug delivery systems using systematic "design of experiments." Part I: Fundamental Aspects"*. Critical Reviews™ in Therapeutic Drug Carrier Systems. 22: 27-105.

[14] Fonner Jr DE, Buck JR, Banker G.S. (1970) *"Mathematical optimization techniques in drug product design and process analysis*". J Pharm Sci 59: 1587-1596.

[15] Shek E, Ghani M, Jones RE. (1980) "*Simplex search in the optimization of capsule formulation"*. J Pharm Sci 69:

[16] Tye H. (2004) "*Application of statistical 'design of experiments' methods in drug discovery*". Drug Discov Today 9: 485-491.

[17] Shrimant N Panaskar, Mahesh H Hampe, Mr. Susheel Singh, Akshata Shivalkar, Pallavi Kadam, "*A Fast & Reliable Liquid Chromatography Mass Spectrometry Method for Determination of Amino acids & Acylcarnitines including Succinyl acetone in dried blood spots for newborn screening*." SSRG International Journal of Medical Science 6.7 (2019): 1-11.