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METHOD DEVELOPMENT AND VALIDATION OF METAXALONE BY UV SPECTROSCOPY

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ABSTRACT

The proposed analytical method was simple, reliable, rapid, sensitive, reproducible and accurate for the estimation of Metaxalone. Metaxalone is a newer skeletal muscle relaxant and used along with rest and physical therapy to decrease muscle pain and spasms associated with strains, sprains or other muscle injuries. ELICO-SL244- Double Beam - UV – Visible spectrophotometer with pair of 10 mm matched quartz cells was used. Drug was dissolved in Methanol and was made further dilutions with Methanol to produce 20 µg/ml. It was scanned in the range of 200-400 nm and it shows constant λ_{max} at 279.5 nm. Stability of the absorbance at their λ_{max} was also checked for up to 4 hours. The optical characteristics such as Beer's law limit (40-200µg/ml), Sandell's Sensivity (0.181228971), correlation coefficient (0.9998), slope (0.00641) and intercept (0.00015) were calculated. The limit of detection was found to be 0.301404286 µg/ml and the limit of quantification was found to be 0.913346321 µg/ml. The drug samples were analyzed by UV spectroscopy using methanol as solvent and the content of drug present in the formulation was found to be 399.17 mg (99.79%). The above method does not suffer from any interference due to common excipients.

KEYWORDS: Metaxalone, UV spectroscopy, Methanol.

INTRODUCTION

Metaxalone[1-2] in humans has not been established, but may be due to general central nervous system depression. Metaxalone has no direct action on the contractile mechanism of striated muscle, the motor end plate or the nerve fiber. Metaxalone is an oxazolidinone with centrally-acting skeletal muscle relaxant properties. Although the exact mechanism through which Metaxalone exerts its effect is largely unknown, it might be due to general central nervous system (CNS) depression. Metaxalone has no direct effect on the contractile mechanisms of striated muscle, the motor end plate, or the nerve fiber. Metaxalone is associated with few side effects and is available as an 800 mg scored tablet.

It is chemically Metaxalone is 5-[(3, 5-dimethylphenoxy) methyl]-2-oxazolidinone. The category of the drug is skeletal muscle relaxant. Metaxalone is white to almost white, odorless crystalline powder in form. Metaxalone is freely soluble in chloroform, soluble in methanol and in 96% ethanol, but practically insoluble in ether or water. The metabolism of Metaxalone involves enzymes CYP1A2 and CYP2C19 in the cytochrome P450 system. Because many medications are metabolized by enzymes in this system, precaution must be taken when administering it with other medications involving the P450 system to avoid interactions.

After through literature survey, the present method was developed as per ICH Guidelines.[3-5] A survey of literature reveals that good analytical methods are not available for the drug like Metaxalone. Even though very few methods of estimation of above drugs are available, many of them suffer from one disadvantage or the other, such as low sensitivity, lack of selectivity and simplicity etc. The existing physicochemical methods are inadequate to meet the requirements. Hence it is proposed to improve the existing methods and to develop new methods for the assay of Metaxalone in pharmaceutical dosage forms adapting different available analytical techniques like UV-Spectrophotometry as shown in **Fig. 1.**
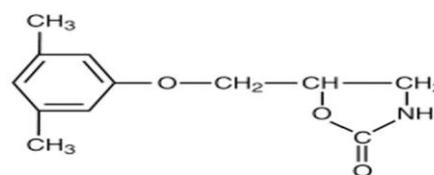

Fig. 1: Structure of Metaxalone.

MATERIAL AND INSTRUMENT[6-8]

Metaxalone was obtained as a gift sample from AUROBINDO Pharmaceuticals Pvt. Ltd., Hyderabad. Skelaxin tablets containing 400 mg of Metaxalone was purchased from local Pharmacy. All other solvents such as Methanol, Potassium Di Hydrogen Phosphate and Sodium Hydroxide were of analytical grade only. Digital Balance (US 300) of model no CYBERLAB-200 was used. ELICO-SL244- Double Beam - UV – Visible spectrophotometer with pair of 10 mm matched quartz cells was used.

UV SPECTROPHOTOMETRIC METHOD[9-11]

Selection of Solvent

The solubility of Metaxalone was determined in a variety of solvents as per Indian Pharmacopoeia standards. Solubility test for Metaxalone was carried out in different polar and non-polar solvents. From the solubility studies methanol was selected as suitable solvent for proposed method.

Preparation of Standard Stock Solution

25 mg of Metaxalone raw material was accurately weighed and transferred into the 25 ml volumetric flask and dissolved in minimum quantity of methanol and made up to 25 ml with methanol.

Selection of λ_{max}

The standard stock solution was further diluted with methanol to get 20 µg/ml concentrations. The solution was scanned between 200-400 nm ranges using methanol as blank. From the UV Spectra 279.5nm was selected as λ_{max} for analysis of Metaxalone. Stability of the Metaxalone in methanol was studied by measuring the same solution at this λ_{max} in different time intervals. It was observed that Metaxalone in methanol was stable for more than 4 hours.

Calibration Graph

In this method, the aliquots of stock solution of Metaxalone (4-20 ml of 250 µg /ml) were transferred in to 25 ml volumetric flask and made up to the mark with Methanol. The absorbance of different concentration solutions were measured at 279.5 nm against blank. The samples were found to be linear from 40-200 µg /ml. The calibration curve was plotted using concentration Vs absorbance. The curve obtained was linear with the concentration range of 40-200 µg /ml as shown in **Fig. 2.**

ASSAY PROCEDURE

Twenty tablets of formulation (SKELAXIN) containing 400mg of Metaxalone was accurately weighed to find out the average weight and powdered. Powdered tablet equivalent to 250 mg of Metaxalone was transferred in to 25 ml volumetric flask, added methanol to dissolve and made up to the volume. Then the solution was sonicated for 15 minutes. After sonication, the solution was filtered through Whatmann filter paper No.41. From the clear solution, further dilution was made to bring a 100 µg /ml using methanol. The prepared solution was measured at 279.5 nm. The amount of Metaxalone was determined by using slope and intercept values from calibration graph as shown in **Table 1.**

RECOVERY STUDIES

From the pre-analyzed formulation, a known quantity of standard solution was added and the contents were mixed well, finally made up to the volume with methanol. Absorbance was measured at 279.5 nm. Amount present was calculated from slope and intercept. Then the % recovery was determined by using the following formula.

$$\%Recovery = \frac{N \sum xy - \sum x \sum y}{N \sum x^2 - (\sum x)^2} \times 100$$

Where,   N   =   Number of observations
           X   =   Amount Added in microgram/ml
           Y   =   Amount recovered in microgram/ml

METHOD VALIDATION[12-15]

Linearity

Drug was dissolved in Methanol and was made further dilutions with Methanol to produce 20 µg/ml. It was scanned in the range of 200-400 nm and it shows constant λ_{max} at 279.5 nm. The absorbance at their λ_{max} was also checked for up to 4 hours. The linearity of the drug Metaxalone was found and its calibration curve was constructed. The optical characteristics such as Beer's law limit (40-200µg/ml), sandell's sensivity (0.181228971), correlation coefficient (0.9998), slope (0.00641) and intercept (0.00015) were calculated as shown in **Table 2** and **Fig. 3.**

Precision - Repeatability

Repeatability of the Metaxalone was carried out by repeating the calibration, formulation, and recovery studies six times. The absorbance was observed repeatedly three times under same experimental conditions. Precision of the method was studied by making repeated analysis of the sample and it was carried out three times in a day and repeated for 3 days. The percentage standard deviation for inter-day and intra-day analysis was found for recovery and assay respectively. Statistical validation for the formulation was also calculated as shown in the **Table 3.**

Ruggedness

The degree of reproducibility of test results obtained by UV-method of Metaxalone was checked by analyzing the drug sample under following test conditions like by using different instruments – Double Beam and single Beam Spectrophotometers, by doing the experiments by different analysts and by doing the experiments with different glassware. To validate and confirm the results, six solutions of Metaxalone were prepared and analysis was carried out as shown in **Table 4.**

Accuracy

To evaluate the accuracy of the method, known amount of pure drug was added to the previously analysed

solution containing Pharmaceutical formulation and the mixture was analysed by the proposed method and the recoveries were calculated. The percentage recovery of Metaxalone sample was found to be 98.24 % - 100.97 %.

Limit of Detection (LOD) and Limit of Quantification (LOQ)
Construction of six sets of calibration graph from the serial dilutions of standard and limit of detection and

limit of quantification was calculated by using the standard deviation of the response and the slope of the calibration curve. The limit of detection was found to be 0.301404286 µg/ml and the limit of quantification was found to be 0.913346321 µg/ml.

Table 1: Quantification of Formulation by UV Method.

S.No	Labeled amount (mg/tab)	Amount found (mg)	Percentage Obtained	Percentage Average	SD	% RSD
1.	400mg	398.79	99.94			
2.	400mg	399.32	99.83			
3.	400mg	396.90	99.22	99.80	0.5641	0.5625
4.	400mg	396.28	99.22			
5.	400mg	401.88	100.47			
6.	400mg	401.88	100.47			

Table 2: Recovery Studies for Metaxalone formulation by UV-Method.

S.No	Concentration used (%)	Amount (µg/ml) Added	Amount (µg/ml) Estimated	Amount (µg/ml) Recovered	% Recovery Obtained	% Recovery Average	SD	% RSD
1.	75	25	74.7	24.87	99.48			
2.	75	25	75.01	25.18	100.72	99.48		
3.	75	25	74.39	24.56	98.24			
4.	100	50	99.66	49.8	99.61			
5.	100	50	99.06	49.23	98.46	99.67	0.9828	0.9867
6.	100	50	100.31	50.48	100.96			
7.	125	75	124.33	74.5	99.33			
8.	125	75	123.86	74.03	98.7	99.66		
9.	125	75	125.56	75.73	100.97			

Table 3: Precision of Metaxalone formulation by UV-Method.

S.No.	Labeled Amount (mg/tab)	Inter Day Amount Found (mg)	Inter Day % Recovered	Intra Day Amount Found (mg)	Intra Day % Recovered
1.	400mg	399.14	98.17	401.56	101.56
2.	400mg	398.67	99.23	400.61	99.8
3.	400mg	399.26	99.67	399.78	98.27
4.	400mg	399.14	98.17	401.56	101.56
5.	400mg	398.67	99.23	400.61	99.8
6.	400mg	399.26	99.67	399.78	98.27
S.D.	-	0.756	1.143	0.834	1.095
% RSD	-	0.815	1.166	1.254	1.125

Table 4: Ruggedness for Metaxalone formulation by UV Method.

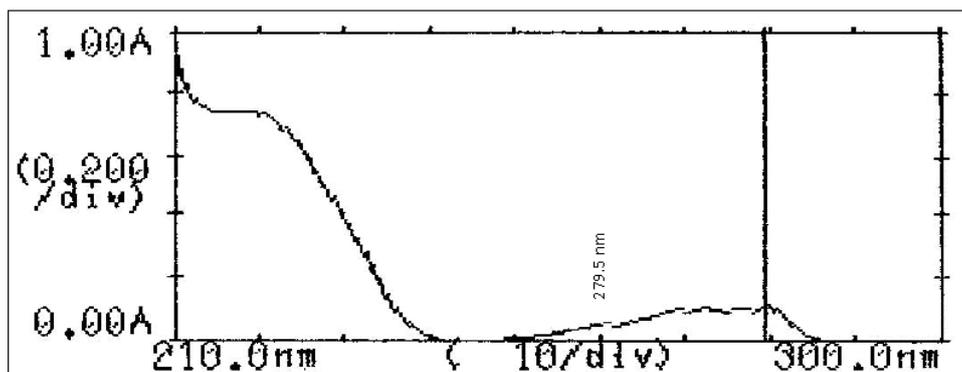| S.No.	Amount Added (mg)	Different Analyst	Different Instrument	Different Days
1.	400mg	399.51	400.60	400.26
2.	400mg	399.32	401.88	401.88
3.	400mg	396.90	401.88	400.12
4.	400mg	396.28	401.56	401.88
5.	400mg	401.88	400.61	401.88
6.	400mg	400.12	398.35	399.47
S.D.	-	0.439	0.256	0.743
% RSD	-	0.622	0.269	0.814

Fig. 2: Ultra Violet Absorption Spectrum of Metaxalone using Methanol.
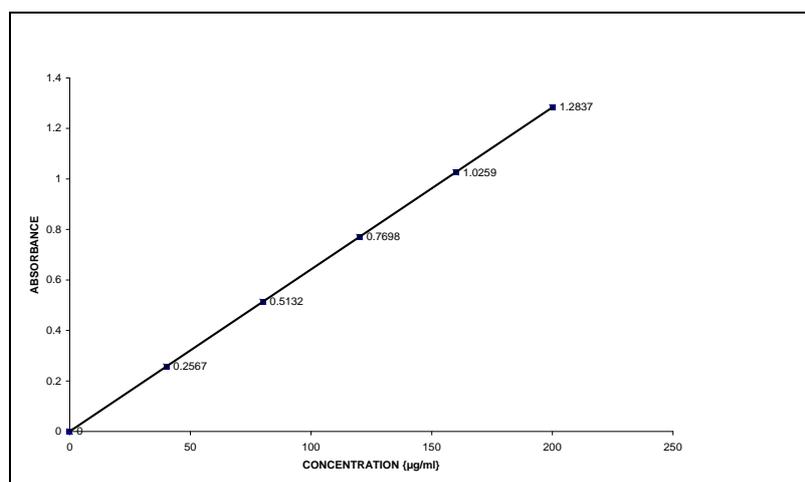
Fig. 3: Calibration Curve of Metaxalone by UV Method using Methanol.

RESULTS AND CONCLUSIONS

Parameters	Method
λ_{max} (nm)	279.,5nm
Beers Law Limit (μg/ml)	40-200μg/ml
Sandell's Sensitivity (μg/cm^2/0.001 A.U)	0.181228971
Molar Absorptivity (L mol^{-1} cm^{-1})	2732.654367
Correlation Coefficient (r)	1.000
Regression Equation (y=mx+c)	Y=0.00641(X) +0.00015
Slope (m)	0.00641
Intercept (c)	0.00015
LOD (μg/ml)	0.301404286
LOQ (μg/ml)	0.913346321

CONCLUSION

The existing physicochemical methods are inadequate to meet the requirements; hence it was proposed to improve the existing methods and to develop new methods for the assay of Metaxalone in pharmaceutical dosage forms adapting different available analytical techniques like UV-Spectrophotometry. The developed method was checked for the performance characteristics and has also been validated. Hence the method can easily and conveniently adopt for the estimation of Metaxalone.

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