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DEVELOPMENT OF ANALYTICAL METHODS FOR THE ESTIMATION OF METAXALONE IN PURE AND SOLID DOSAGE FORMS BY UV- SPECTROPHOTOMETRIC AND RP-HPLC METHODS

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ABSTRACT

The present study was designed to develop a simple, validated UV-Spectrophotometric method and a RP-HPLC method for the analysis of Metaxalone in bulk and pharmaceutical dosage forms. The fast and reliable UV-Spectrophotometric Method was carried out using the values measured at 280 nm in methanol for Metaxalone. Calibration graph was constructed at the wavelength of determination was linear in the concentration range of using 40-200 µg/ml for Metaxalone and the correlation coefficient was found to be 0.9998. Precision study showed that the % RSD was within the range of acceptable limits (< 2), and the % Recovery was found to be in the range of 98.24 % - 100.97 %. The LOD and LOD were found to be 0.301404286 µg/mL and 0.913346321 µg/mL respectively. A precise and accurate RP-HPLC method was developed by isocratic operation of mobile phase. The best stationary phase was determined as C_{18} column, 5 µm, 250 mm × 4.6 mm. Mobile phase was optimized to obtain a fast and selective separation of the drug. Flow rate was 1.00 mL/min, Wavelength was set at 210 nm and the volume of each injection was 20 µL. An isocratic Phosphate buffer (pH 5): Acetonitrile: Methanol mobile phase at the ratio of 40:40:20 gave the best separation and resolution. The proposed methods were accurate, precise, sensitive, and linear over a wide range of concentration of Metaxalone. The developed methods have been validated as per ICH guidelines. The validated methods were successfully applied to the determination of Metaxalone in bulk and pharmaceutical dosage forms.

Key Words:- Metaxalone, UV-Spectrophotometric, RP-HPLC, ICH guidelines.

INTRODUCTION

Analytical chemistry deals with methods for determining the chemical composition of samples of matter (Skoog *et al.*, 2005). Instrumental/Physico-chemical methods of chemical analysis have now become the backbone of experimental chemistry. These methods may be used by the analytical chemist to save time and to avoid chemical separation or to obtain increased accuracy. Instrumental methods are based on the theory of relation between the concentration of chemicals/ drugs and the corresponding physico-chemical properties of chemical components being analyzed (Beckett *et al*., 2002).

Metaxalone is a muscle relaxant used to relax muscles and relieve pain caused by strains, sprains and other musculoskeletal conditions. Chemically Metaxalone is 5-[(3, 5-dimethylphenoxy) methyl]-1, 3-oxazolidin-2-one (Fig 1.). It is a white to almost white, odourless

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crystalline powder freely soluble in chloroform, soluble in methanol, 96% ethanol and water. Molecular formula of Metaxalone is $C_{12}H_{15}NO_3$ and molecular weight is 221.2524.

The mechanism of action of metaxalone in humans has not been established, but may be due to general central nervous system depression. It has no direct action on the contractile mechanism of striated muscle, the motor end plate or the nerve fibre. Metaxalone is metabolized by the liver and excreted in the urine as unidentified metabolites. The impact of age, gender, hepatic, and renal disease on the pharmacokinetics of metaxalone has not been determined. In the absence of such information, metaxalone should be used with caution in patients with hepatic and/or renal impairment and in the elderly. The most common adverse effects of metaxalone are drowsiness and dizziness. There may occasionally also be gastrointestinal irritation and gastrointestinal bleeding has been reported rarely. Other effects that have occurred are headache, overstimulation, and rarely sensitivity reactions including skin rashes, petechiae, ecchymoses, urticaria and pruritus; very rarely, angioedema or anaphylactic reactions may occur. Some patients taking metaxalone have developed jaundice and liver damage suspected to be caused by the drug. After over dosage there may be gastrointestinal disturbances, drowsiness, dizziness, headache, malaise, and sluggishness followed by marked loss of muscle tone, hypotension and respiratory depression (Martindale, 2009).

From literature survey on estimation of Metaxalone various methods have been reported that include UV-Spectroscopic method (Priyadarshini *et al.,* 2011), RP-HPLC method (Nagavalli *et al.,* 2010 and Cacae *et al.,* 2004), LC-MS method (Nirogi *et al.,* 2006), RP-UPLC method (Rakshit *et al.,* 2012) and HPTLC method (Milindkumar *et al.,* 2011). In present work Metaxalone was analyzed by UV spectroscopy using methanol as solvent and a newer RP-HPLC method was developed for both bulk drug and formulation using Buffer: Acetonitrile: Methanol in the ratio of 40:40:20.

MATERIALS AND METHODS

UV Spectrophotometric Method

Selection of solvent

The solubility of Metaxalone was determined in a variety of solvents as per Indian Pharmacopoeia standards. The solubility of Metaxalone was determined in a variety of solvents, sample approximately (10 mg) was taken in the test tube and various solvents were added to checking the solubility. The solvents used were distilled water, NaOH (0.1M), HCL (0.1M), Methanol, Acetonitrile, Acetone, Ethanol, Chloroform, Diethyl ether, Benzene, Toluene, Carbon Tetra Chloride, Ethyl Acetate, Isopropranol, DMF and Dichloromethane.

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.

Quantification of formulation

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.

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.

$$\%\text{Recovery} = \frac{N\sum XY - \sum X \sum Y}{N\sum X^2 - (\sum X)^2} \times 100$$

Where, N = Number of observations; X = Amount Added in microgram/Ml and

Y = Amount recovered in microgram/mL

Limit of detection (LOD) and Limit of quantification (LOQ)

Construct six sets of calibration graph from the serial dilutions of standard. The limit of detection and limit of quantification was calculated by using the standard deviation of the response and the slope of the calibration curve.

Repeatability

Repeatability of the Metaxalone was carried out by repeating the calibration, formulation, recovery studies six times.

Ruggedness

The degree of reproducibility of test results obtained by UV-method of Metaxalone was checked by analyzing the drug sample under the test conditions such as by using different instruments – Double Beam and single Beam Spectrophotometers, by different analysts and with different glass wares.

To validate and confirm the results, six solutions of Metaxalone were prepared and analysis was carried out.

RP-HPLC Method

Materials

Metaxalone was obtained as a gift sample from AUROBINDO Pharmaceuticals Pvt.Ltd., Hyderabad. The drug sample received was authenticated by its melting point. Skelaxin tablets containing 400 mg of Metaxalone was purchased from local market. HPLC-grade methanol, HPLC-grade acetonitrile, potassium dihydrogen phosphate and sodium hydroxide were from Qualigens India Pvt. Ltd., and Loba Chemie India Ltd. HPLC grade water used in the analysis was prepared by reverse osmosis and passed through a 0.45µm millipore filter (Millipore Company, USA) before use. A pH 5.0 phosphate buffer (10 mM), was prepared by dissolving 27.208 g of potassium dihydrogen phosphate in HPLC-grade water in a 1000 mL beaker, then the volume was made up to the mark and pH was adjusted with Orthophosphoric acid.

Equipment

Instruments: HPLC system (SHIMADZU AX – 200 DIGITAL BALANCE) with double reciprocating plunger pump, SPD – IOA_{VP} SHIMADZU UV – Vis detector with column oven was employed. LC–10 ATVP SHIMADZU solvent deliver module software was used for data acquisition and processing. For UV-Spectrophotometric analysis of Metaxalone was performed by using Shimadzu UV – Visible spectrophotometer (Shimadzu, UV-1700; Cuvets: 1cm quartz cells) with Silicon Photodiode detector.

Analytical column: Metaxalone was analyzed by reverse-phase HPLC using Octadecyl Silane C18 column (250 mm × 4.6 mm, 5.0µ) column with isocratic mode of operation.

Determination of appropriate UV wavelength

A suitable wavelength was required for determination of Metaxalone. The appropriate wavelength for the detection of the drug in mobile phase was determined by wavelength scanning over the range of 200–400 nm with a Shimadzu UV – Visible spectrophotometer. The λ_{max} was found as 280 nm.

Chromatographic conditions

Two organic solvents (acetonitrile and methanol), different volume fractions of a filtered and degassed methanol and acetonitrile and phosphate buffer with pH (5.0) at concentrations of 10 mM were selected as mobile phase at 210 nm of wavelength. This wavelength was selected because it is a UV maximum and provides the sensitivity needed for quantitation of the low drug concentration in pharmaceutical dosage forms. The column temperature was maintained at Ambient. The mobile phase was pumped at different flow rate of 1.0 mL/min with 20 µL injection volume.

Preparation of Standard solution

An accurately weighed quantity of Metaxalone (10 mg) was transferred to a 50 mL volumetric flask; approximately 5 mL of the methanol was added and dissolved. The solution was brought to volume by methanol and properly mixed to obtain a final concentration of 200 mg/mL. The prepared stock solution was stored in a glass vial. From this stock solution, standard solutions were freshly prepared prior to analysis.

HPLC method validation

Method validation was performed on the best determined stationary phase *i.e.* C18 column, 5 µm, 250 mm × 4.6 mm.

Linearity and Calibration

Five solution of 20-100µg/mL concentration was prepared by transferring the standard solution in 10mL volumetric flask and making up the volume upto the mark with methanol. Each concentration level was prepared in triplicate and analyzed three times. Calibration curves were constructed by plotting the concentration of compounds versus peak area response. The linearity was evaluated by the least square regression method.

System suitability studies

The system suitability studies carried out as specified in USP. The parameters like column efficiency, tailing factor, Asymmetric Factor Capacity Factor were calculated.

Recovery studies

To ensure the accuracy of the method, recovery studies were performed standard addition method at 50%, 75% and 100% levels of drugs cons to the pre-analyzed sample and they were re-analyzed. Results obtained are showed in Table 2.

Quantification of metaxalone in formulation

Each tablet containing 400 mg of metaxalone was taken. The average weight of each tablet were found and crushed to powder. The powder was weighed equivalent to 250 mg of Metaxalone and was transferred into a 50 ml volumetric flask, sufficient quantity of methanol was added and was sonicated for few minutes and made up to the mark with methanol. The solution was filtered through Whatmann filter paper No.41.From this clear solution further dilution was made with mobile phase and produced 50 µg/ml solutions. The peak area measurements were done by injecting the sample (50 µg/ml) six times and the amount of Metaxalone calculated from the respective calibration curve.

RESULT AND DISCUSSION

UV-Spectroscopic Studies

From the solubility studies methanol was selected as suitable solvent for proposed method. 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 this is shown in Fig 2. Stability of the absorbance at their λ_{max} was also checked for upto 4 hours. The linearity of the drug Metaxalone was found; its calibration curve was constructed and is shown in Fig 3. 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 and shown in Table1.

To evaluate the accuracy of the method, known amount of pure drug was added to the previously analyzed solution containing Pharmaceutical formulation and the mixture was analyzed by the proposed method and the recoveries were calculated. The percentage recovery of Metaxalone sample was found to be 98.24 % - 100.97 %. These values were given in Table 2.

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 which are tabulated in Table 3. Statistical validation for the formulation was also calculated, which are shown in the Table 4.

RP-HPLC Studies

An effort has been made to develop a simple, specific and accurate method for the estimation of Metaxalone in Formulation.

The λ_{max} of Metaxalone in mobile phase was found at 280nm. The different combination of mobile phase was employed for the analysis. Optimization of the method for the mobile phase of Buffer: Acetonitrile: Methanol (40:40:20) was carried by changing the various pH and flow rates. The chromatogram for optimized method was shown in Fig 4. From the above studies the mobile phase consisting of Buffer: Acetonitrile: Methanol (40:40:20) at pH 5.0 and at flow rate 1 ml/min was selected from the system suitability parameter which were within the limit and it is shown in Table 5.

Various concentrations of raw material such as 20-100µg/ml of Metaxalone were injected and the chromatograms were recorded and the calibration curve for the same concentration was plotted and are shown in Fig 5. The optical characters such as Beers law limits (40-200µg/ml), molar extinction co-efficient (18202820363), sandell's sensitivity (2.121213 E.08), Correleation co-efficient (0.9997), slope (41553.5485) and intercept (32629.2381) were calculated and is shown in Table 6. The results were found to be satisfactory.

The system suitability parameters such as Theoretical Plates, Tailing Factor and Asymmetric Factor were calculated (Table 7). The parameters were found to be satisfactory as per guidelines.

Repeatability was performed for same concentration six times to the formulation and the amount present was found to be 399.17 mg and 399.98 mg respectively. The values are shown in Table 8. Precision was confirmed by repeatable injection of the formulation Fig 6.

Accuracy was confirmed by recovery studies (Fig 7.) by adding known amount of pure drug to the previously analyzed formulation and the mixture was analyzed by the proposed method was found to be 98.04 %-101.57 %. The values are given in the Table 9. The proposed method was validated and found that the excipients and additives did not interfere the developed method.

All the above parameters combined with the simplicity and ease of operation ensures that the application of proposed method in the assay of drug in pharmaceutical dosage forms. Thus the proposed RP-HPLC method may also be applied for the estimation of Metaxalone raw materials and Pharmaceutical dosage forms. Statistical validation for the formulation was also calculated, which are shown in the Table 10 and the comparative results for the proposed two methods are shown in Table11.

Table 1. Optical characteristics of Metaxalone by UV method

Sl. No.	Parameters	Method
1	λ_{max}(nm)	280 nm
2	Beers law limit (µg/ml)	40-200µg/ml
3	Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.181228971
4	Molar absorptivity (L mol^{-1} cm^{-1})	2732.654367
5	Correlation coefficient (r)	0.9998
6	Regression equation (y=mx+c)	Y=0.00641(X) +0.00015
7	Slope(m)	0.00641
8	Intercept(c)	0.00015
9	LOD (µg/ml)	0.301404286
10	LOQ (µg/ml)	0.913346321

Table 2. Recovery studies for metaxalone formulation by UV-method

Sl. No.	Concentration used for estimation (%)	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	% RSD
1	75	49.83	25	74.7	24.87	99.48	0.9828	0.9867
2	75	49.83	25	75.01	25.18	100.72		
3	75	49.83	25	74.39	24.56	98.24		
4	100	49.83	50	99.66	49.8	99.61		
5	100	49.83	50	99.06	49.23	98.46		
6	100	49.83	50	100.31	50.48	100.96		
7	125	49.83	75	124.33	74.5	99.33		
8	125	49.83	75	123.86	74.03	98.7		
9	125	49.83	75	125.56	75.73	100.97		

Table 3. Intra-day and Inter-day analysis of Metaxalone formulation and its recovery studies by UV-method

Sl. No.	Assay of Metaxalone Formulation		Recovery Studies	
	*Interday (Amount found in mg)	*Intraday (Amount found in mg)	*Interday (% Recovered)	*Intraday (% Recovered)
1	399.14	401.56	98.17	101.56
2	398.67	400.61	99.23	99.8
3	399.26	399.78	99.67	98.27
S.D.	0.756	0.834	1.143	1.095
% RSD	0.815	1.254	1.166	1.125

*****Mean of six observations.

Table 4. Statistical validation for Metaxalone formulation by UV-method

Validation for Assay of Metaxalone formulation		Validation for Recovery Studies	
Labeled amount (mg/tab)	400	**Labeled amount (mg/tab)**	400
***Amount found (mg)**	399.17	***Amount found (mg)**	398.40
***Percentage obtained**	99.79	***% Recovery**	99.60
S.D.	0.564	**S.D.**	0.982
% RSD	0.5625	**% RSD**	0.986

* Mean of six observations.

Table 5. System suitability parameters for the optimized chromatogram by RP-HPLC method

Sl. No.	Parameters	Metaxalone
1	Tailing factor	1.27
2	Asymmetrical factor	1.53
3	Theoretical plates	5222
4	Theoretical Plate Per unit length	348.13

Table 6. Optical characteristics of metaxalone by RP-HPLC method

Sl. No.	Parameters	Method
1	λ_{max}(nm)	280nm
2	Beers law limit (µg/ml)	20-100
3	Sandell's sensitivity (µg/cm^2/0.001 A.U)	2.121213E-.08
4	Molar absorptivity (L mol^{-1} cm^{-1})	18202820363
5	Correlation coefficient (r)	0.9997
6	Regression equation (y=mx+c)	Y=41553.5485+32629.2381
7	Slope(m)	41553.5485
8	Intercept(c)	32629.2381
9	LOD (µg/ml)	0.320525186
10	LOQ (µg/ml)	0.971288443
11	Standard error of mean of Regression line	34028.99659

Table 7. System suitability parameters for the optimized chromatogram by RP-HPLC method

Sl. No.	Parameters	Value
1	Tailing factor	1.27
2	Asymmetrical factor	1.53
3	Theoretical plates	5222
4	Theoretical Plate Per unit length	348.13

Table 8. Quantification of formulation: Skelaxin by HPLC method

Sl. No.	Expected amount (mg/tab)	Amount found (mg)	Percentage obtained	Average (%)	S.D.	% RSD
1	400	401.72	100.43	99.99	0.3656	0.3656
2		400.80	100.20			
3		399.76	99.94			
4		399.20	99.80			
5		397.60	99.40			
6		400.80	100.20			

Table 9. Recovery studies for metaxalone formulation by RP-HPLC Method

Sl.No.	Concentration used for estimation (%)	Amount Present (µg/ml)	Amount Added (µg/ml)	Amount Estimated (µg/ml)	Amount Recovered (µg/ml)	% Recovery	S.D.	% RSD
1	50	24.99	25	49.5	24.51	98.04	1.21	1.209
2	50	24.99	25	49.86	24.87	99.48		
3	50	24.99	25	50.19	25.2	100.8		
4	75	24.99	50	75.23	50.24	100.48		
5	75	24.99	50	74.47	49.48	98.96		
6	75	24.99	50	75.43	50.44	100.88		
7	100	24.99	75	101.17	76.18	101.57		
8	100	24.99	75	99.48	74.49	99.32		
9	100	24.99	75	100.5	75.51	100.68		

Table 10. Statistical Validation for Metaxalone formulation by RP-HPLC Method

Validation for Assay of Metaxalone formulation		Validation for Recovery Studies	
Labeled amount (mg/tab)	399.98	**Labeled amount (mg/tab)**	400
***Percentage obtained**	99.90	***% Recovery**	100.05
S.D.	0.440	**S.D.**	1.21
% RSD	0.441	**% RSD**	1.20

* Mean of six observations

Table 11. Comparative results of Metaxalone by UV and RP-HPLC Methods

Sl. No.	Method	Formulation	Labeled Amount(mg/tab)	* Amount found (mg)	% Obtained	S.D.	%S.D
1	UV	SKELAXIN	400	399.17	99.79	0.5953	0.5966
2	RP-HPLC		400	399.98	99.99	0.3656	0.3656

* Mean of six observations

Fig 1. Structure of Metaxalone

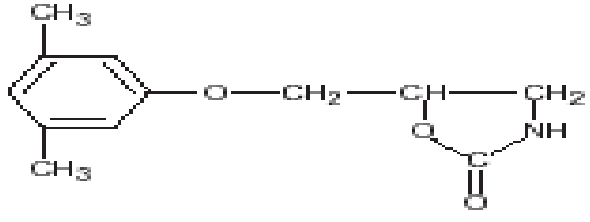


Fig 2. Ultra violet absorption spectrum of Metaxalone using methanol

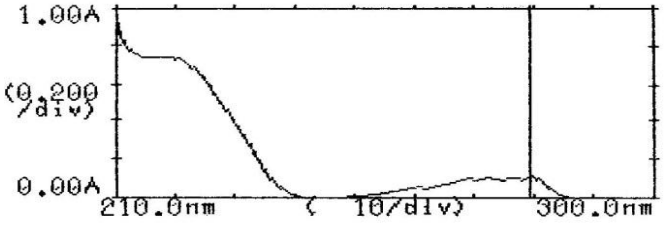


Fig 3. Calibration curve of Metaxalone by UV-method using methanol

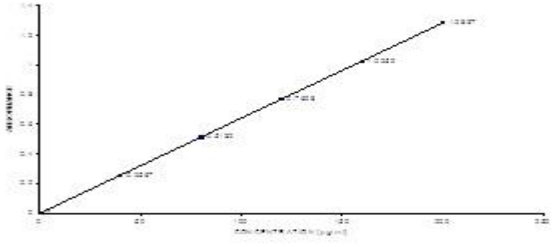
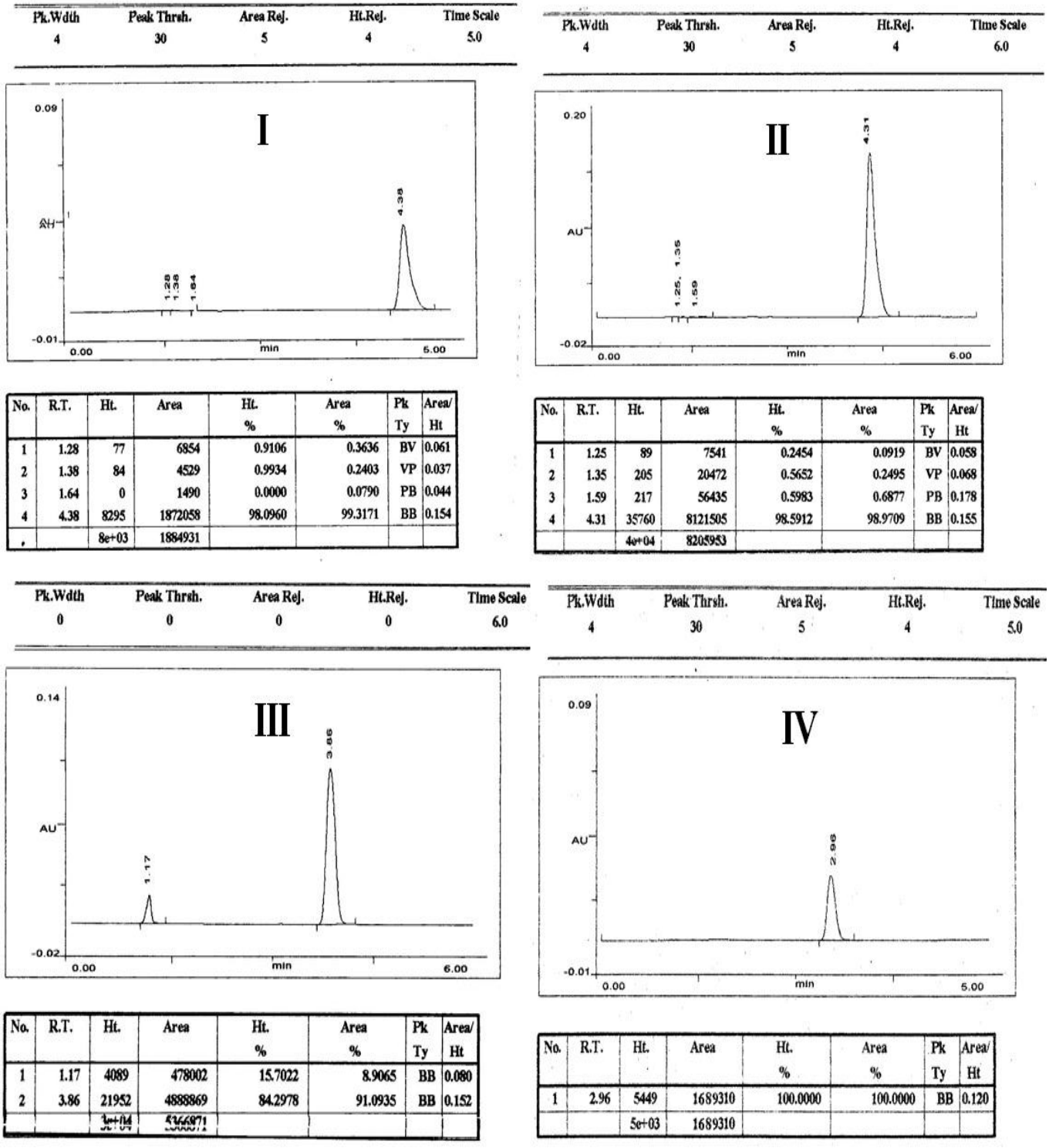


Fig 4. Optimization of chromatogram by changing the pH and the flow rate of mobile phase in Buffer: Acetonitrile: Methanol (40:40:20); I-pH 4, II- pH 5, III-pH 6, IV-pH 5 and flow rate 0.8 mL/min, V- pH 5 and flow rate 1.0 mL/min, VI- Optimized Chromatogram for Metaxalone in mobile phase.



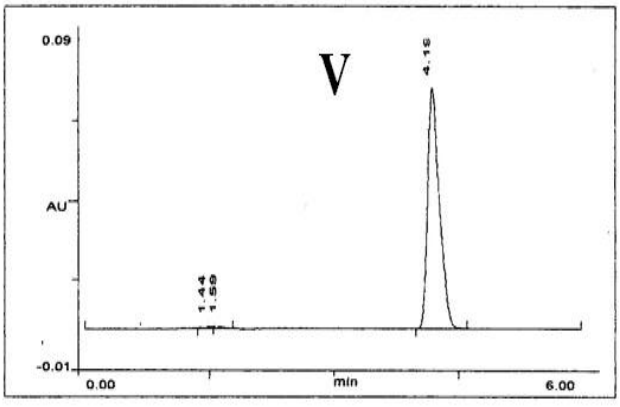
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.28	77	6854	0.9106	0.3636	BV	0.061
2	1.38	84	4529	0.9934	0.2403	VP	0.037
3	1.64	0	1490	0.0000	0.0790	PB	0.044
4	4.38	8295	1872058	98.0960	99.3171	BB	0.154
		8e+03	1884931				

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.25	89	7541	0.2454	0.0919	BV	0.058
2	1.35	205	20472	0.5652	0.2495	VP	0.068
3	1.59	217	56435	0.5983	0.6877	PB	0.178
4	4.31	35760	8121505	98.5912	98.9709	BB	0.155
		4e+04	8205953				

No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.17	4089	478002	15.7022	8.9065	BB	0.080
2	3.86	21952	4888869	84.2978	91.0935	BB	0.152
		[illegible]	[illegible]				

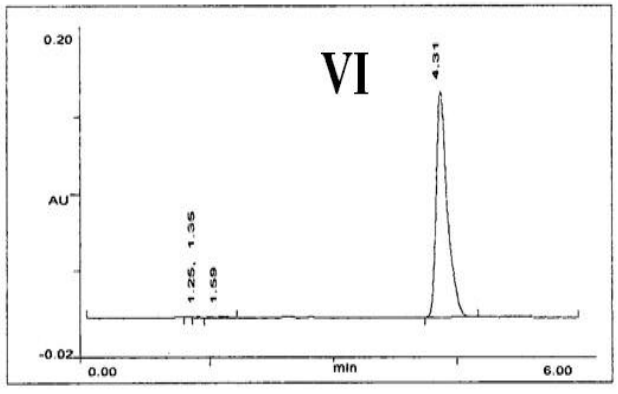
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	2.96	5449	1689310	100.0000	100.0000	BB	0.120
		5e+03	1689310				

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.44	125	24521	0.7362	0.6087	BV	0.134
2	1.59	147	22035	0.8658	0.5470	VB	0.102
3	4.19	16706	3981960	98.3979	98.8443	BB	0.163
		2e+04	4028516				

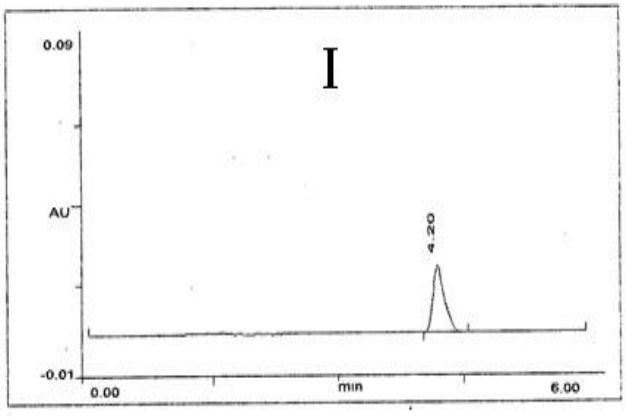
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.25	89	7541	0.2454	0.0919	BV	0.058
2	1.35	205	20472	0.5652	0.2495	VP	0.068
3	1.59	217	56435	0.5983	0.6877	PB	0.178
4	4.31	35760	8121505	98.5912	98.9709	BB	0.155
		4e+04	8205953				

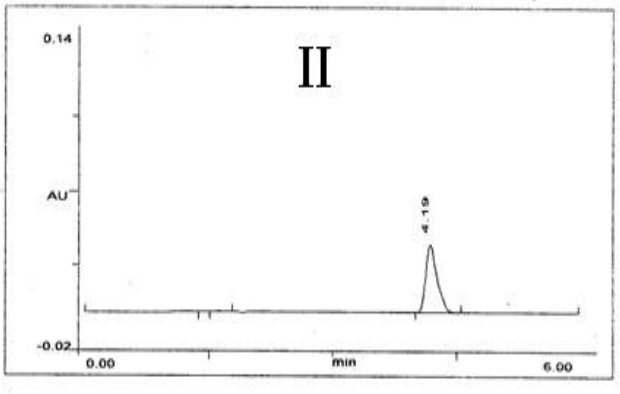
Fig 5. Linearity Chromatogram of Metaxalone I-20 µg/ml, II-40 µg/ml, III-60µg/ml, IV-80 µg/ml, V-100 µg/ml, VI-Calibration curve of Metaxalone by RP-HPLC method

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	4.20	4588	1057399	100.0000	100.0000	BB	0.157
		5e+03	1057399				

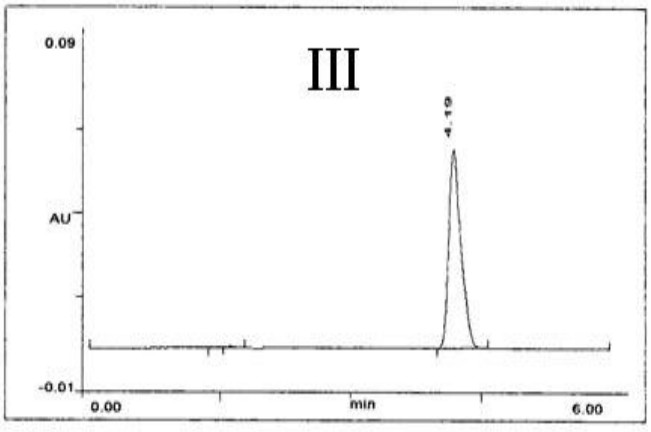
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	4.19	13021	1687414	98.2643	98.8569	BB	0.161
		1e+04	1687414				

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0

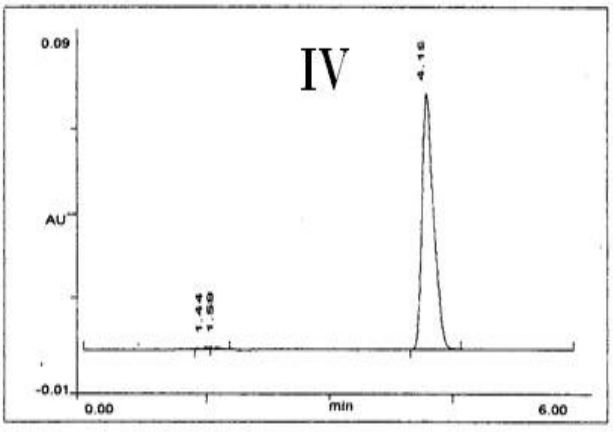
III



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	4.19	13021	3069400	98.2643	98.8569	BB	0.161
		1e+04	3104892				

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0

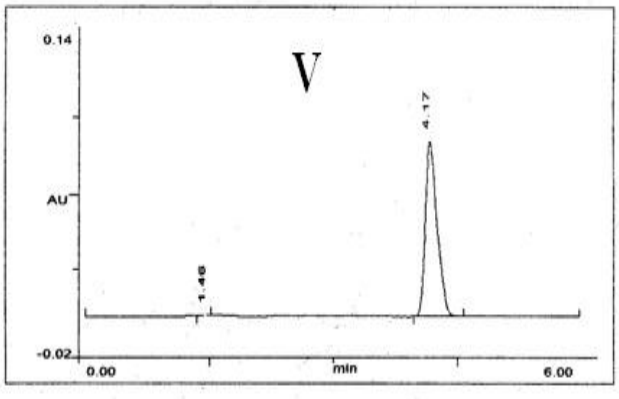
IV



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.44	125	24521	0.7362	0.6087	BV	0.134
2	1.59	147	22035	0.8658	0.5470	VB	0.102
3	4.19	16706	3981960	98.3979	98.8443	BB	0.163
		2e+04	4028516				

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0

V



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.46	0	7865	0.0000	0.1615	BB	0.103
2	4.17	20203	4863365	100.0000	99.8385	BB	0.164
		2e+04	4871230				

VI

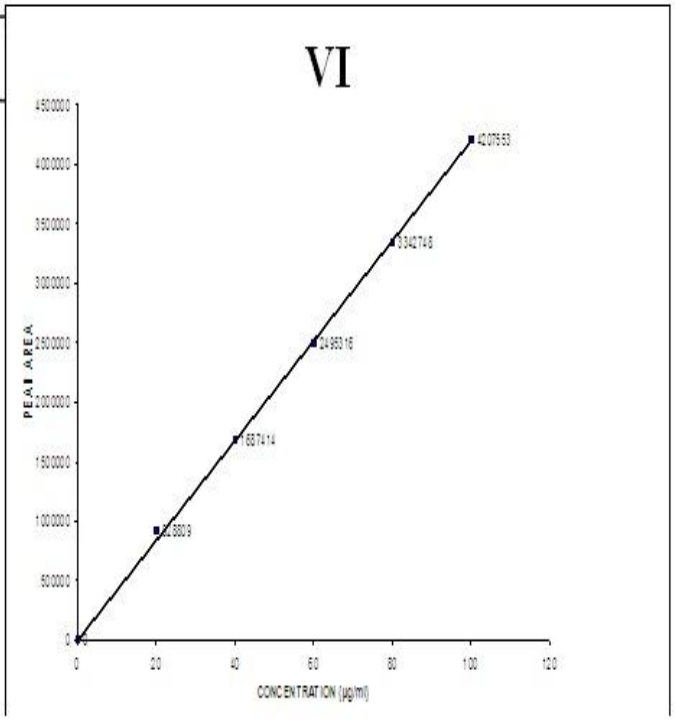
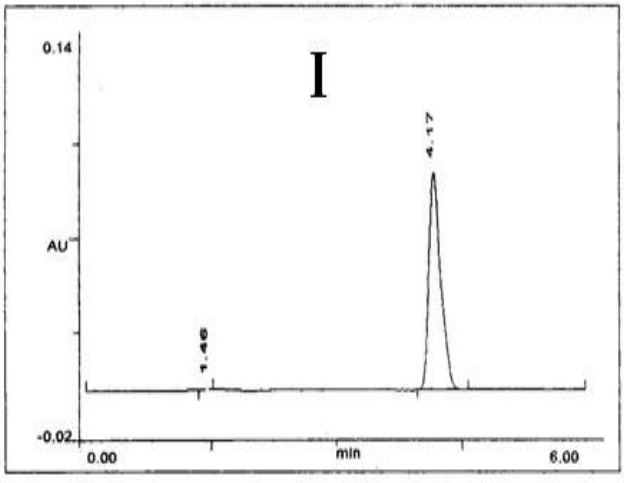


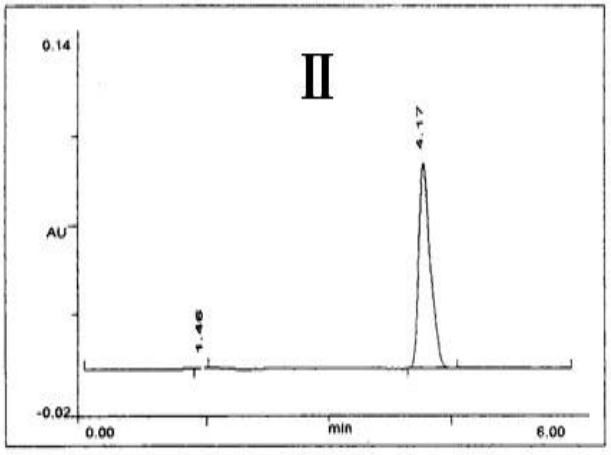
Fig 6. Chromatogram for analysis of Metaxalone formulation Repeatability (I-VI)

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0



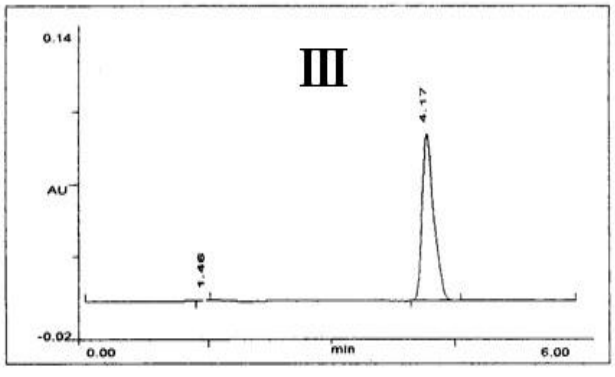
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.46	0	7865	0.0000	0.1615	BB	0.103
2	4.17	20203	2120100	100.0000	99.8385	BB	0.164
		2e+04	2120100				

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0



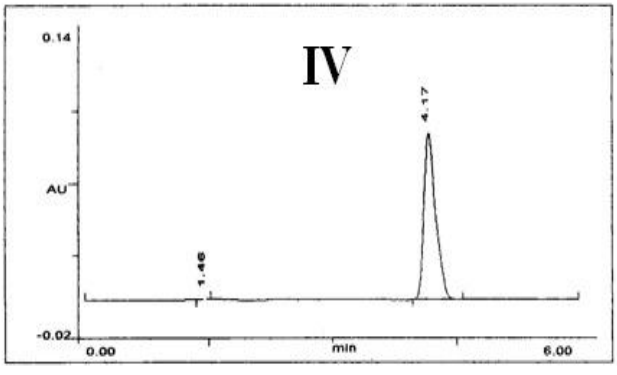
No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.46	0	7391	0.0000	0.1614	BB	0.103
2	4.17	20203	2114500	100.0000	99.9386	BB	0.164
		2e+04	2114500				

Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0

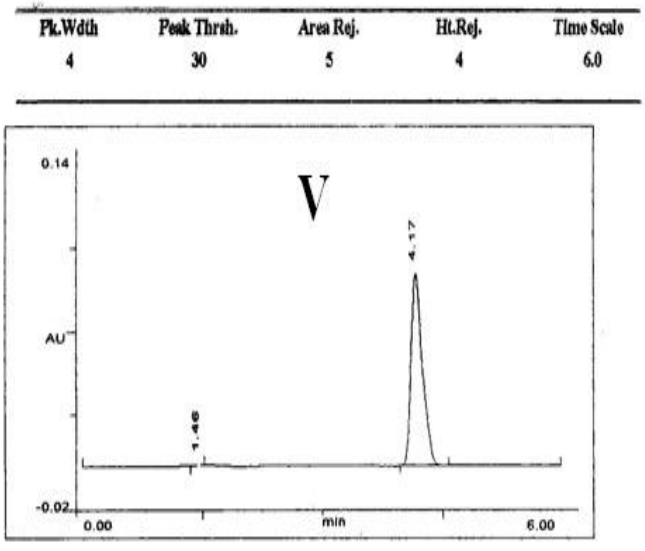


No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.46	0	7519	0.0000	0.1984	BB	0.105
2	4.17	20203	2109459	100.0000	99.8386	BB	0.165
		2e+04	2109459				

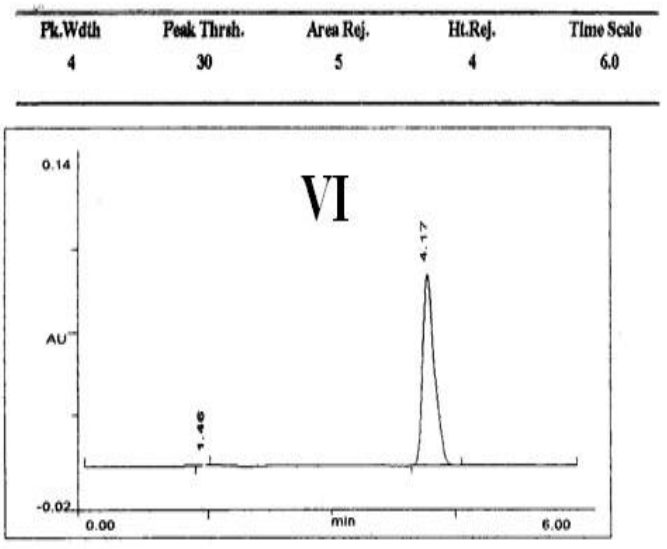
Pk.Wdth	Peak Thrsh.	Area Rej.	Ht.Rej.	Time Scale
4	30	5	4	6.0



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.46	0	7865	0.0000	0.1695	BB	0.101
2	4.17	20203	2110100	100.0000	99.7195	BB	0.166
		2e+04	2110100				

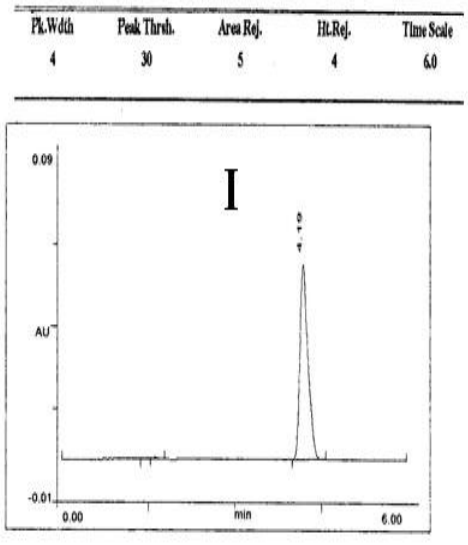


No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.46	0	7391	0.0000	0.1614	BB	0.103
2	4.17	20203	2098794	100.0000	99.9386	BB	0.164
		2e+04	2098794				

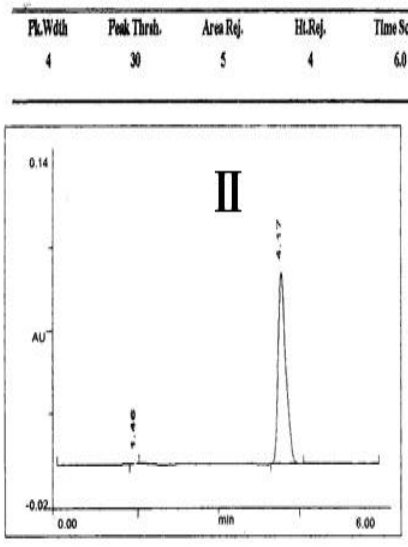


No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.46	0	7865	0.0000	0.1615	BB	0.103
2	4.17	20203	2116541	100.0000	99.8385	BB	0.164
		2e+04	2116541				

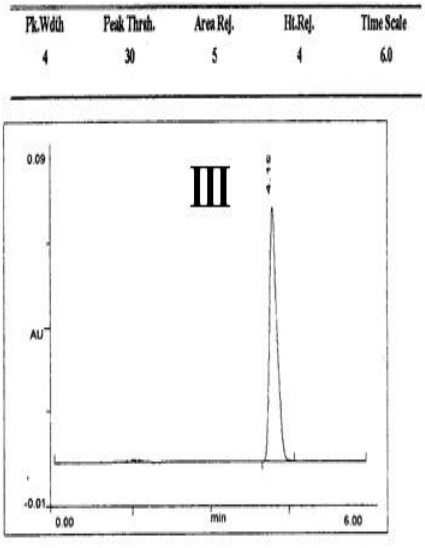
Fig 7. Chromatogram for the recovery of Metaxalone formulation (I-III)



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	4.19	13021	2089624	98.2643	98.8569	BB	0.161
		1e+04	2089624				



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	1.46	0	7865	0.0000	0.1615	BB	0.103
2	4.17	20203	3158964	100.0000	99.8385	BB	0.164
		2e+04	3158964				



No.	R.T.	Ht.	Area	Ht. %	Area %	Pk Ty	Area/ Ht
1	4.19	16706	4236918	98.3979	98.8443	BB	0.163
		1e+04	4236918				

CONCLUSION

Metaxalone is a newer skeletal muscle relaxant and is used along with rest and physical therapy to decrease muscle pain and spasms associated with strains, sprains or other muscle injuries. The proposed analytical methods of Simple UV-Spectroscopic method and RP-HPLC method were found to be simple, reliable, rapid, sensitive, reproducible and accurate for the estimation of Metaxalone. 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%). A newer RP-HPLC method was developed for both bulk drug and formulation. This proposed method given reliably assay results with short analysis time (<5.0mins) using the mobile phase of Buffer: Acetonitrile: Methanol in the ratio of 40:40:20. The content of drug present in the formulation was found to be 399.98 mg (99.99%). Both the methods do not suffer from any interference due to common excipients. Therefore, the proposed methods could be successfully applied to estimate commercial Pharmaceutical products containing Metaxalone. Thus the above studies and findings will enable the quantification of the drug for future investigation in the field of analytical chemistry. Among the established analytical methods, RP-HPLC method was found to be more précised and accurate. The %RSD calculated for RP-HPLC method was very less when compared to UV method. Hence RP-HPLC method can be applied for regular analysis of Metaxalone from bulk drug and its dosage forms.

REFERENCES

Beckett AH and Stenlake JB. Practical Pharmaceutical Chemistry- Part B, 4th ed., CBS publishers and Distributors, New Delhi, 2002, 279-298, 158-316.

Cacae J, Reilly EE, Amann A. Comparison of dissolution of metaxalone tablets (Skelaxin) using USP apparatus 2 and 3. *AAPS Pharm Sci Tech*, 5, 2004, E6.

http://www.medicinenet.com/metaxalone/article.htm Searched on 20th May 2013.

Martindale. The Complete Drug Reference, 36th ed., Pharmaceutical Press, London, Chicago, 2009, 1897.

Milindkumar PR, Vishal VB, Savita SY, Toufik SM and Janhavi RR. Validated HPTLC Method for simultaneous estimation of Diclofenac Potassium and Metaxalone in bulk drug and formulation. *Pharmacie Globale (IJCP)*, 2(12), 2011, 1-4.

Nagavalli D, Sankar ASK, Anandkumar K, Vetrichelvan T, Nalaji M. Estimation of metaxalone in bulk and in tablet dosage form by RP-HPLC. *Res J Pharmacy Technol*, 3, 2010, 409–411.

Nirogi RV, Kandikere VN, Shukla M, Mudigonda K, Shrivastava W, Datla PV. Quantification of metaxalone in human plasma by liquid chromatography coupled to tandem mass spectrometry. *J Anal Toxicol*, 30, 2006, 245–251.

Priyadarshini J, Saraswathi D, Ajithadas A, Jerad Suresh A. Hydrotropic solubilisation technique of metaxalone by area under curve and second order derivative spectroscopy. *Res J Pharmacy Technol*, 4, 2011, 72–74.

Rakshit KT and Mukesh CP. Development of a stability-Indicating RP-UPLC Method for rapid determination of Metaxalone and its degradation products in solid oral dosage form; *Sci pharm*, 80, 2012, 356-366.

Skoog DA and West DM. Fundamentals of Analytical Chemistry, 8th ed., Thomson Asia Pvt. Ltd., Singapore, 2005, 314.