http://www.e-journals.net

ISSN: 0973-4945; CODEN ECJHAO
E-Journal of Chemistry
2011, **8(S1),** S439-S447

Development and Validation of Stability Indicating RP-HPLC Method for the Determination of Metaxalone in Bulk and its Pharmaceutical Formulations

PRAFULLA KUMAR SAHU[*], M. MATHRUSRI ANNAPURNA[§] and SAHOO DILLIP KUMAR

[*]Department of Pharmaceutical Analysis
Roland Institute of Pharmaceutical Sciences, Khodasinghi, Berhampur-760010, Orissa, India
[§]Department of Pharmaceutical Analysis & QA, GITAM Institute of Pharmacy, GITAM University, Rushikonda, Visakhapatnam, Andhra Pradesh-530045, India
Department of Pharmaceutical Analysis, Avanthi Institute of Pharmaceutical Sciences Cherukupally, Bhogapuram, Vizianagaram, Andhra Pradesh-531162, India
kunasahu1@rediffmail.com

Received 30 March 2011; Accepted 7 June 2011

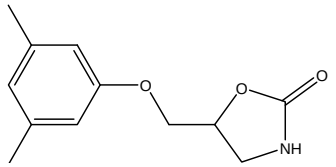
Abstract: A stability indicating reverse phase HPLC method was developed for the determination of metaxalone, a skeletal muscle relaxant, present in bulk and its pharmaceutical formulations using gliclazide as the internal standard (I.S). A hypersil ODS C18 column (250 x 4.6 mm, packed with 5 micron) in an isocratic mode with mobile phase Acetonitrile: phosphate buffer 3.6 (50:50%v/v) was used at a flow rate of 0.8 mL/ min and effluent was monitored at 225 nm. The assay exhibited a linear dynamic range of 0.6-100 µg/mL. Acceptable precision and accuracy were obtained for concentrations over the standard curve range. The retention times were 5.13 min and 9.08 min for metaxalone and IS respectively. The extraction recovery of metaxalone from pharmaceutical dosage form (tablets) was >97% and the calibration curve was linear (r^2 = 0.999) over the entire linear range. The method had an accuracy of >98% and LOD and LOQ of 0.2 µg/mL and 0.6 µg/mL respectively. The specificity of the proposed method was performed whereby metaxalone undergoes different stress conditions like oxidation, reduction, photolysis, acid and alkaline hydrolysis.

Keywords: Metaxalone, RP-HPLC, Stability studies, Gliclazide

Introduction

Chemically, metaxalone (Figure 1) is 5-[(3, 5- dimethylphenoxy) methyl]-2-oxazolidinone. It is indicated as an adjunct to rest, physical therapy and other measures for the relief of

discomforts associated with acute, painful musculoskeletal conditions[1]. The mechanism of action of metaxalone 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 end plate, or the nerve fiber. There is very limited or inconsistent data regarding the effectiveness and safety of metaxalone[3]. Metaxalone is one of the commonly used muscle relaxant therapies for acute low back pain[4].



(5-[(3,5- Dimethylphenoxy) methyl]-2-oxazolidinone)

Figure 1. Chemical structure of metaxalone

Literature survey revealed that no HPLC methods are reported for the determination of metaxalone till date. One method is available for the quantification of metaxalone in human plasma by liquid chromatography coupled to tandem mass spectrometry[5]. Metaxalone has been used as an internal standard for few analytical methods[6-7]. Present study involves development and validation[8-9] of a stability indicating[10] RP-HPLC method for the estimation of metaxalone in bulk and its pharmaceutical formulations.

Experimental

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC10AT and LC10AT VP series HPLC pumps, with a 20 µL Injection of sample loop (manual) and SPD 10A VP UV-Visible Detector. The output signal was monitored and integrated using Shimadzu CLASS-VP Version 6.12 SP1 software. Hypersil ODS C_{18} (46 mm x 25 cm, 5 mm) column was used for the separation. The pH of the solution was adjusted by using digital pH meter, model DI 707 (Digisun electronics, Hyderabad, India).

Standards and chemicals

Metaxalone and gliclazide gift samples were obtained from aurobindo pharma (Hyderabad, India) and Ranbaxy Laboratories Limited (Mohali, India). Flexura tablets (Sun Pharma) containing 400 mg of metaxalone, were purchased from local market. Purified water was prepared using a Millipore Milli-Q (Bedford, M.A., USA) water purification system. Acetonitrile of HPLC grade was purchased from Burdick and Jackson (Muskagon, MI, USA), ammonium acetate of A.R. grade and formic acid of A.R. grade were purchased from local suppliers.

Preparation of mobile phase

Phosphate buffer 3.6 was prepared by dissolving 0.900 g of anhydrous disodium hydrogen phosphate and 1.298 g of citric acid monohydrate in sufficient water to produce 1000 mL. Acetonitrile of HPLC grade was mixed with the phosphate buffer 3.6 in a ratio of 50: 50. It was then sonicated for about 30 min and filtered through 0.45 micron membrane filter which was used for analysis of metaxalone.

Preparation of standard drug solutions

Stock solution of metaxalone was prepared by dissolving 25 mg of metaxalone in 25 mL of volumetric flask containing 20 mL acetonitrile. The solution was sonicated for about 20 min and then made up to volume with acetonitrile. Daily working standard solutions of metaxalone

was prepared by suitable dilution of the stock solution with appropriate mobile phase. Similarly stock solution and working standard solutions of internal standard was also prepared.

Chromatographic conditions

The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 0.8 mL/min. The eluents were monitored at 225 nm. Although the λ_{max} of metaxalone in the mobile phase is 272 nm, but good resolution, peak area were resulted at 225 nm. The column temperature was maintained ambient throughout the experiment. The identification of the separated metaxalone and gliclazide were confirmed by running the chromatograms of the individual compounds under identical conditions.

Procedure for tablets

20 Tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 25 mg of metaxalone was extracted with acetonitrile in a 25 mL volumetric flask using ultra sonicator. This solution was filtered through Whatman No 1 filter paper. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously determined. An aliquot (20 µg/mL) of the internal standard was added to the sample solution prior to the dilution. All determinations were carried out in six replicates. The represented data were shown in (Table 1 & 2).

Table 1. Recovery studies

Conc. in µg/mL	Retention time		Ratio of AUC (Drug/I.S) (n = 6)	Accuracy (n = 6)	%RSD
	Drug	I.S			
20	5.132	9.075	0.8067±0.007	104.66±1.108	1.059
40	5.132	9.081	1.5446±0.006	108.22±0.502	0.4640
80	5.132	9.083	2.6409±0.024	106.82±0.931	0.974

Values are expressed in Mean ±SD, (n=6)

Table 2. Recovery study from formulation of metaxalone

AUC Ratio (Drug/I.S) (40µg/ml) (n = 6)	Labeled amount	Calculated amount	Accuracy (n = 6)	%RSD
1.338±0.001	400 mg	389 mg	97.25±0.427	0.437

Values are expressed in Mean ±SD, (n=6)

Method development and optimization

During the method development, top priority was given for the complete separation of metaxalone and forcibly degraded products under the stress conditions such as acid, base, oxidation, reduction and neutral degradation. The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase and composition of mobile phase. Several mobile phases were tested until good resolution, retention time and tailing obtained. Mixture of 20 mM potassium dihydrogen phosphate buffer (pH 3.6) and acetonitrile (ACN) in the proportions of 40:60, 45:55, 50:50, 60:40 and 65:35 (v/v) were tested as a mobile phase with hypersil ODS C_{18} column. Increasing the composition of organic modifier decrease in retention time, the peak shape of drug was poor and shoulder peak was observed (40:60 v/v Buffer: ACN). Decreasing the composition of organic modifier increase in retention time (60:40v/v Buffer: ACN). The mobile phase composition of 55:45 v/v buffer: ACN, resolution, retention time was good but tailing factor is high. The method was optimized with the mobile phase

composition of acetonitrile and phosphate buffer 50:50 (v/v). Buffer molarity of 10, 20 and 50 mM was tested. There were no significant changes in the chromatographic response and peak shape with change in buffer molarity. A buffer molarity of 20 mM was selected for further analysis.

After several trials, the method was optimized as a mixture of 20 mM potassium dihydrogen phosphate buffer (pH 3.6) and acetonitrile (50:50 v/v), at a flow rate of 0.8 mL/min, at 225 nm for run time of 12 min. These chromatographic conditions achieved satisfactory resolution, retention tailing for metaxalone. The Figure 2 & 3 shows that chromatogram of metaxalone and in formulations respectively.

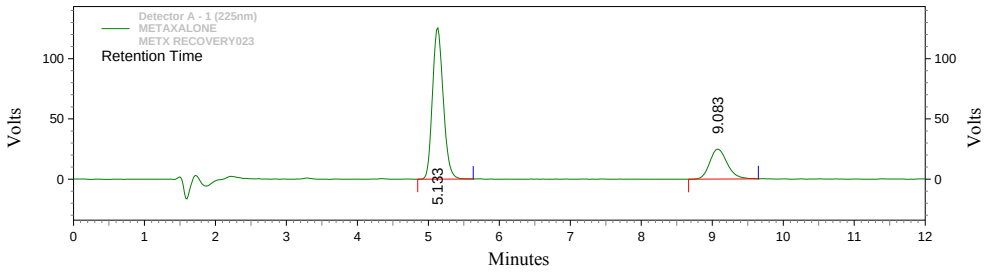


Figure 2. Typical chromatogram of metaxalone

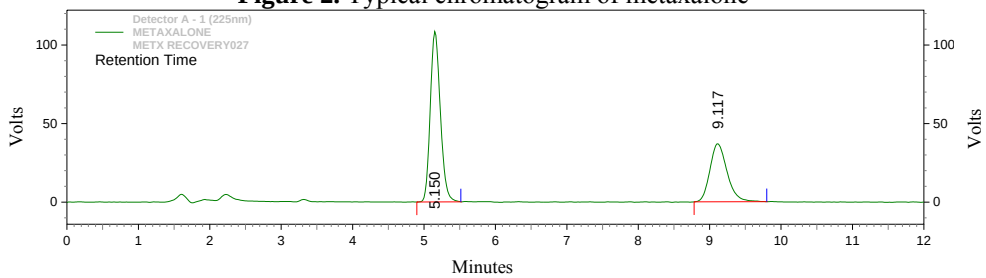


Figure 3. Chromatogram of metaxalone in formulation

Method validation

Method was validated accordance to ICH guidelines[9], for system suitability, linearity, precision, accuracy, limit of detection, limit of quantification, robustness, specificity and solution stability. For system suitability, six replicates of standard sample were injected and studied the parameters like plate number (N), tailing factor (k), resolution (R) and relative retention time (α), HETP, capacity factor (k^1), plates per meter and peak symmetry of samples.

Linearity

The linearity of this method was evaluated by linear regression analysis, which was calculated by least square method. Calibration standards were prepared by spiking required volume of working standard (100 µg/mL) solution into different 10 mL volumetric flasks and volume made with methanol to yield concentrations of 0.6, 1, 2, 5, 10, 20, 50 and 100 µg/mL. To the above solutions 20 µg /mL of gliclazide (Internal Standard) was added and the final volume was made up to the mark. A 20 µL aliquot was injected in to the analytical column. The resultant peak areas of the drugs were measured. Calibration curve was plotted between peak area of drug against concentration of the drug. These results show there was an excellent correlation between peak area and analyte concentration. The linearity results are presented in Table 3 and Figure 4.

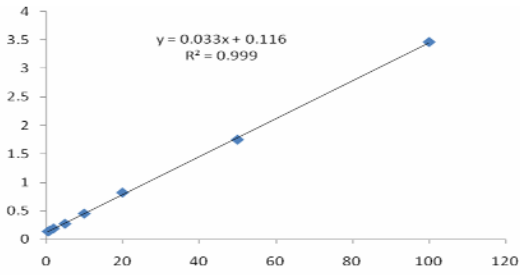


Figure 4. Linearity graph of metaxalone

Table 3. Linearity of metaxalone

Conc. in µg/mL	Retention Time		Mean Ratio of AUC (Drug/I.S) (n = 3)	Accuracy	Parameters
	Drug	I.S			
0.6	5.137	9.083	0.134652	94.20	Slope (m) = 0.033
1	5.137	9.083	0.148708	99.11	
2	5.137	9.083	0.187265	107.97	Intercept
5	5.147	9.083	0.270595	93.69	(c) = 0.116
10	5.133	9.083	0.448408	100.72	Standard deviation
20	5.137	9.083	0.817255	106.25	(SD) =0.6515
50	5.133	9.083	1.747002	98.84	%RSD =0.711
100	5.153	9.083	3.461369	101.37	

Note: Retention time of metaxalone is 5.150 min and IS (Gliclazide) is 9.117 min

Intra-day and Inter-day precision and accuracy

Precision and accuracy was studied by quality control samples of standard solutions covering low, medium and high concentrations (0.6, 5, 50 µg/mL) of linearity range were prepared and injected. Peak areas of three replicated injections of each concentration were measured. Intra-day precision was studied by six replicate measurements at three concentration levels in the same day. Inter-day precision was conducted during routine operation of the system over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evaluation revealed that relative standard deviation of the drug at different concentration levels for six injections was less than 2. Precision and accuracy data were shown in Table 4 and 5.

Table 4. Intra-day precision and accuracy of metaxalone

Conc. in µg/mL	Retention time		Ratio of AUC (Drug/I.S) (n = 6)	Accuracy (n = 6)	%RSD
	Drug	I.S			
0.6	5.147	9.083	0.13413	91.56±0.6515	0.711
5	5.137	9.083	0.269798	93.21±0.7867	0.786
50	5.133	9.083	1.747238	98.86±0.4070	0.411

Values are expressed in Mean ±SD, (n=6)

Table 5. Inter-day precision and accuracy of metaxalone

Conc. in µg/mL	Retention time		Ratio of AUC (Drug/I.S) (n = 6)	Accuracy (n = 6)	%RSD
	Drug	I.S			
0.6	5.135	9.075	0.13487	95.33±0.6515	0.831
5	5.131	9.081	0.27148	94.23±0.7867	0.808
50	5.132	9.083	1.74880	98.95±0.4070	0.493

Values are expressed in Mean ±SD, (n=6)

Limits of detection and quantification

Limit of detection (LOD) and limit of quantification (LOQ) was calculated based on the standard deviation of the response and the slope.

Specificity

The specificity of the method was demonstrated through forced degradation studies (Table 6) conducted on the sample using acid, alkaline, oxidative, reductive and photolytic degradations. The sample was exposed to these conditions and the main peak was studied for the peak purity, thus indicating that the method effectively separated the degradation products from the pure active ingredient.

Table 6. Degradation study of metaxalone

Stress Conditions	Retention time		Ratio of AUC (Drug/I.S) (n = 3)	%Drug Recovered (n = 3)	%RSD
	Drug	I.S			
Standard Drug	5.133	9.083	3.4613	101.37±0.65	0.711
Oxidation	5.083	8.917	3.0394	87.33±1.791	2.051
Reduction	5.083	8.915	3.2659	95.45±1.208	1.266
Alkali	5.083	8.908	2.5194	74.01±1.459	1.972
Acid	5.083	8.925	3.2044	94.77±2.195	2.316
Photolytic (UV)	5.083	8.932	3.3214	97.13±0.525	0.541

Values are expressed in Mean ±SD, (n=3)

Alkali degradation

About 10 mg of metaxalone pure drug was accurately weighed and transferred to 10 mL volumetric flask. To this 1ml of 0.1 N NaOH was added and kept aside for one hour and volume was made up with mobile phase. Then from this stock solution, 10 µg/mL solution was prepared. To one mL of this drug solution one mL of internal standard solution (20 µg/mL) was mixed and 20 µL of this mixture was injected to obtain chromatograms Figure 5.

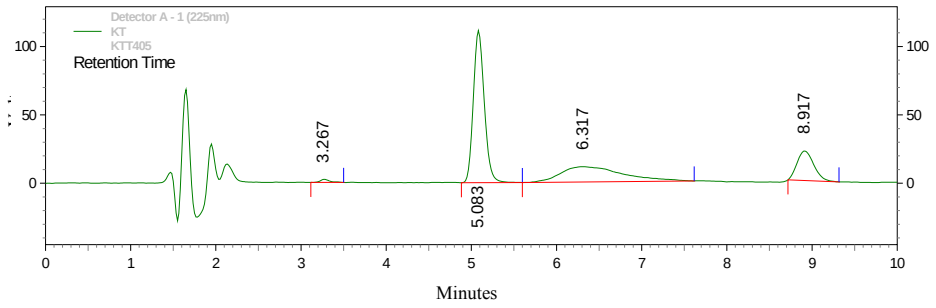


Figure 5. Degradation of metaxalone in alkali conditions

Note: Retention time of metaxalone is 5.083 min and IS (Gliclazide) is 8.917 min

Acid degradation

About 10 mg of metaxalone pure drug was accurately weighed and transferred to 10 mL volumetric flask. To this 1 mL of 0.1 N HCL was added and kept aside for 1 h and volume was made up with mobile phase. Then from this stock solution, 10 µg/mL solution was prepared. To one ml of this drug solution one mL of internal standard solution (20 µg/mL) was mixed and 20 µL of this mixture was injected to obtain chromatograms (Figure 6).

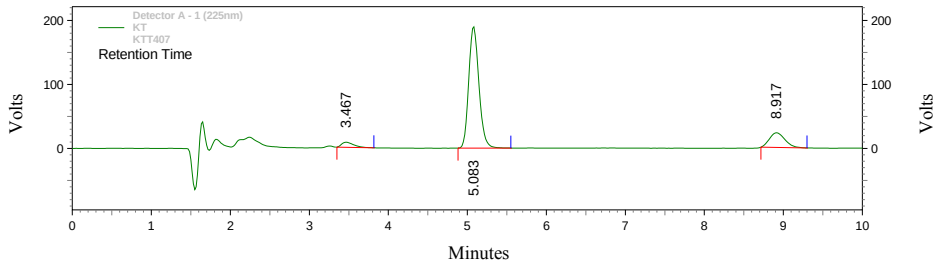


Figure 6. Degradation of metaxalone in acid conditions

(Retention time of metaxalone is 5.083 min and IS (Gliclazide) is 8.917 min)

Oxidation degradation

About 10 mg of metaxalone pure drug was accurately weighed and transferred to 10 mL volumetric flask. To this 1 mL of 3%w/v of hydrogen peroxide was added and kept aside for two hrs and volume was made up with mobile phase. Then from this stock solution, 10 µg/mL solution was prepared. To one mL of this drug solution one mL of internal standard solution (20 µg/mL) was mixed and 20 µL of this mixture was injected to obtain chromatograms (Figure 7).

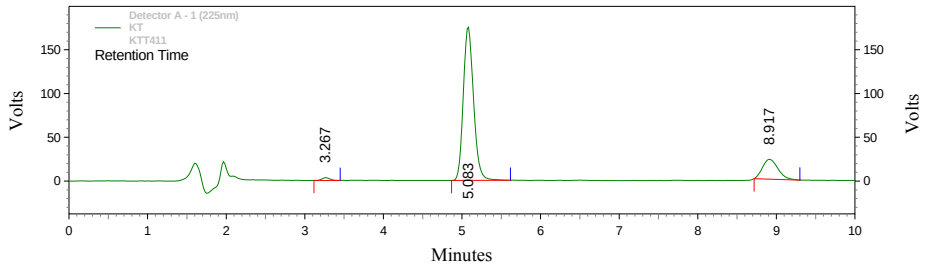


Figure 7. Degradation of metaxalone in oxidative conditions

(Retention time of metaxalone is 5.083 min and IS (Gliclazide) is 8.917 min)

Reduction degradation

About 10 mg of metaxalone pure drug was accurately weighed and transferred to 10 mL volumetric flask. To this 1 mL of 0.1N HCl and small amount of zinc were added and volume was made up with mobile phase. The solution was heated for 1 h at 60 ^{0}C. Then from this stock solution, 10 µg/mL solution was prepared. To one mL of this drug solution one mL of internal standard solution (20 µg/mL) was mixed and 20 µL of this mixture was injected to obtain chromatograms (Figure 8).

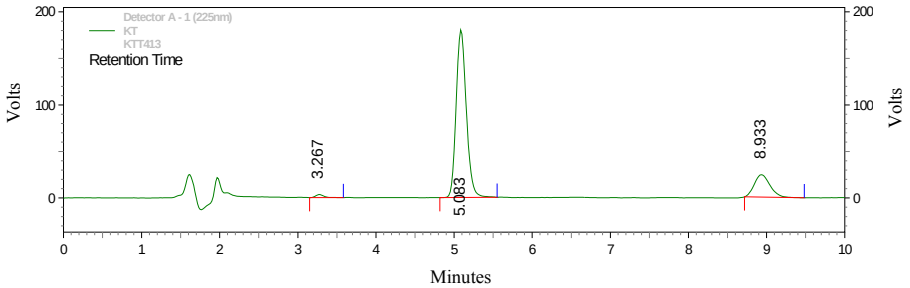


Figure 8. Degradation of metaxalone in reductive conditions

(Retention time of metaxalone is 5.083 min and IS (Gliclazide) is 8.933 min)

Photolytic degradation

About 10 mg of metaxalone pure drug was accurately weighed and transferred to 10 mL volumetric flask and volume was made up with mobile phase. This solution is then kept under U.V for 48 h at 254 nm. Then from this treated solution, 10 µg/mL solution was prepared. To one mL of this drug solution one mL of internal standard solution (20 µg/mL) was mixed and 20 µL of this mixture was injected to obtain chromatograms (Figure 9).

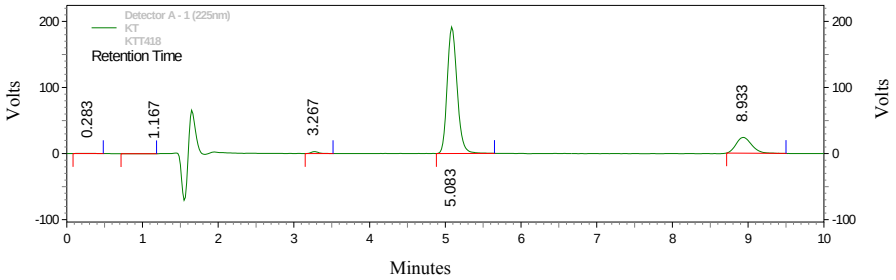


Figure 9. Degradation of metaxalone in photolytic conditions

(Retention time of metaxalone is 5.083 min and IS (Gliclazide) is 8.933 min)

Robustness

Robustness of the method was done by changing slight variation in the parameters like mobile phase, flow rate and pH of the mobile phase. Present method didn't show any significant change when the critical parameters were modified. The tailing factor for metaxalone was always less than 2.0 and the components were well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method, as well as carrying the experiment at room temperature may conclude that the method conditions were robust.

Results and Discussion

The linearity of this method was evaluated by linear regression analysis, which was calculated by least square method and the drug was linear in the concentration range of 0.6-100 µg/mL. The percent accuracy for Interday/ intraday Precision and Accuracy studies were found to be 95.33 to 98.95 and 91.56 to 98.86 respectively. Limit of detection was found to be 0.2 µg/mL and limit of quantification was found to be 0.6 µg/mL. In recovery studies the % accuracy were found to be 104.66 to 106.82 and from tablet formulation 97.25 which are well in the acceptance range. In specificity studies, the % drug recovered under different stress conditions were found to be 74.01 (Alkali Degradation), 94.77 (Acid Degradation), 87.33 (Oxidation Degradation), 95.45 (Reduction Degradation) and 97.13 (Photolytic Degradation).

The chromatographic method was optimized by changing various parameters, such as pH of the mobile phase, organic modifier and buffer used in the mobile phase. Retention of metaxalone has more dependence on pH of the mobile phase when compared to IS. The separation of peaks was also dependent on pH of the buffer and the percentage of acetonitrile. Under the presently prescribed conditions, the % recoveries of metaxalone were found to be within 94.20 to 107.97. This indicates that commonly used excipients in pharmaceutical formulation were not interfering in the proposed method.

Conclusion

The method provides high % recoveries of metaxalone and hence is very useful for determination of metaxalone in pharmaceutical dosage forms. The differences of less than 2.0% for both intra- and inter-day data reflect the precision of the method. The observation of % C.V less than 2.0 for both intra- and inter-day measurements also indicates high degree of precision. In the present method, a hypersil ODS C18 column has been used and the buffer pH in the mobile is 3.6, which is within the limits (pH 2-8) specified by the manufacturers. In the present method, we have established a linearity range of 0.6-100 µg /mL; this linearity range covers all the strengths of metaxalone. Hence this method can be applied for quantifying the low levels of metaxalone in bulk and pharmaceutical dosage forms.

From the specificity studies we found that metaxalone is more susceptible to alkali and oxidation. The % drug recovered under these conditions was found to be less as compared to other stress conditions. The chromatograms indicate that the peaks of the degradants obtained under stress conditions are well resoluted and were not interfering in the proposed method. Hence this can be useful as a stability indicating method for the determination of metaxalone in bulk and pharmaceutical dosage forms.

Acknowledgment

Prafulla Kumar Sahu is thankful to Roland Institute of Pharmaceutical Sciences, Berhampur, Orissa, India, for providing all types of facilities for the research and to Dr. M. E. Bhanoji Rao and my Ph.D supervisor, Dr. M. M. Annapurna for their valuable suggestions and help.

References

1. Portenoy R K, *J Pain Symptom Manage.*, 2000, **19(1)**, 16-20.
2. Waldman H J, *J Pain Symptom Manage.*, 1994, **9(7)**, 434-441.
3. Chou R, Peterson K and Helfand M, *J Pain Symptom Manage.*, 2004, **28(2)**, 140-175.
4. Toth P E and Urtis J, *Clinical Therapeutics*, 2004, **26(9)**, 1355-1367.
5. Nirogi R V, Kandikere V N, Shukla M, Mudigonda K, Shrivastava W and Datla P V, *J Anal Toxicol.*, 2006, **30(4)**, 245-51.
6. Mistri H N, Jangid A G, Pudage A, Gomes N, Sanyal M and Shrivastav P, *J Chromatogr B*, 2007, **853(1)**, 320-332.
7. Mistri H N, Jangid A G, Pudage A and Shrivastav P, *J Chromatogr B*, 2008, **864(1-2)**, 137-148.
8. FDA, Analytical Procedures and Methods Validation: Chemistry, Manufacturing and Controls; Federal Register (Notices), 2000, **65(169)**, 52, 776–777.
9. The European Medicines Agency, Pre-Authorization Evaluation of Medicines for Human Use. ICH Q2(R1): Validation of Analytical Procedures: Text and Methodology, EMEA/410412/2007: London, 2009.
10. Shah D A, Bhat K K, Mehta R S, Baldania S L and Gandh T R, *Indian J Pharm Sci.*, 2008, **70(6)**, 754-760.