PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.9, No.3, pp 171-180, 2016

Stability Indicating High Performance Liquid Chromatographic Method for the Estimation of Carisoprodol in Bulk and in Tablet Dosage form

D. Murali* and C. Rambabu

Department of Biochemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India-522510

Abstract: Simple and sensitive stability indicating HPLC method was developed for the determination of Carisoprodol in bulk and in their tablet formulation. Effective chromatographic separation was achieved on Zorbax Eclipse plus C18 (250 x 4.6 mm; 5 µm particle size) analytical column through isocratic elution mode. The mobile phase composed of 10mM potassium dihydrogen orthophosphate-methanol-acetonitrile in the ratio of 60:20:20 (*v/v/v*). Detection was performed at 240 nm. Analytical performance of the proposed method was statistically validated with respect to linearity, precision, accuracy, robustness, ruggedness, specificity, detection and quantification limits. The linearity range was 1-30 µg/ml with correlation coefficient 0.9995. Carisoprodol was also subjected to acid, base, oxidative and dry heat stress degradation conditions. The degradation products obtained were well resolved from the carisoprodol. The validated stability indicating HPLC method was successfully applied to the analysis of Carisoprodol in their pharmaceutical tablets.
Key words : Carisoprodol, stability indicating, HPLC, tablet dosage forms.

Introduction:

Carisoprodol[1-3], chemically known as 2-(carbamoyloxymethyl)-2-methylpentyl] N-propan-2-yl carbamate, is a skeletal muscle relaxant belonging to monocarboxylic acids and derivatives class of organic compounds. The mechanism of carisoprodol is not known exactly. It acts as a sedative by blocking pain sensations between the nerves and the brain. Along with rest and physical therapy, carisoprodol is used in the treatment of injuries and painful musculoskeletal conditions.

The United States Pharmacopoeia recommended a liquid chromatography method with a refractive index detector for the determination of carisoprodol in tablet dosage forms[4]. Few reports are found in the literature for carisoprodol assay. They include liquid chromatography-tandem mass spectrometry[5,6], gas chromatography[7,8], high-performance thin-layer chromatography[9] and homogeneous immunoassay[10]. All these analytical techniques have been employed for carisoprodol determination in biological samples such as urine & serum of equine and urine & plasma of human[5-10]. Furthermore the reported methods are cumbersome and require sophisticated equipment.

For the routine analysis of the drug in bulk and pharmaceutical dosage forms in quality control laboratories, relatively uncomplicated and cost effective methods like UV/visible spectrophotometry, spectrofluorometry or high performance liquid chromatography with UV detector is required. Ravi *et al.,* reported three extractive spectrophotometric methods for the quantification of carisoprodol in pure and in pharmaceutical formulations[11]. The methods are based on formation of colored chloroform extractable ion-pair

complexes of carisoprodol with dyes like bromocresol green, bromothymol blue and bromophenol blue in acidic medium. However, these methods suffer from one or the other disadvantage such as extraction of ion-pair complex, poor sensitivity, unstable color and rigid experimental conditions.

An UV-HPLC method for the assay of carisoprodol and its impurities viz 2-methyl-2-propylpropane-1, 3-diyl dicarbamate and N-isopropyl -2-methyl-2-propyl-3-hydroxy propyl carbamate was presented by Rohith *et al.,*[12]. The reported UV-HPLC method has some drawbacks in terms of precision, accuracy, retention time (16.855 minutes) and run time (50 minutes). Inaddtion, the gradient mode of elution will increases the use of solvents and the method is more concentrated on the characterization of impurities rather than the assay of carisoprodol. The main aim of the present investigation is to develop and validate a simple, sensitive, cost effective, selective and reproducible stability indicating HPLC method with UV detector for quantitative determination of carisoprodol in bulk and tablet dosage forms.

Experimental:

Instrumentation:

Separation and quantization of carisoprodol was performed on a High Pressure Liquid Chromatography (Shimadzu HPLC class LC series) equipped with two LC-10 AT, VP pumps and variable wavelength programmable UV detector. The HPLC data were processed using LC solution soft ware.

Chemicals and solvents:

HPLC grade acetonitrile and methanol was purchased from Merck India Limited, Mumbai, India. Analytical grade potassium dihydrogen phosphate**,** hydrochloric acid, sodium hydroxide and hydrogen peroxide were from Sdfine-Chem limited, Mumbai, India. Milli-Q-water was used throughout the process

Chromatographic conditions:

Chromatographic separation was carried out on a Zorbax Eclipse plus C18 (250 x 4.6 mm; 5 µm particle size) analytical column by using a mixture of 10 mM potassium dihydrogen orthophosphate: methanol: acetonitrile (60:20:20 *v/v/v*) at a flow rate of 0.7 ml/min as the mobile phase. The wavelength for the UV detection was 240 nm. The column temperature was maintained at 27±1 °C and the injection volume was 10 µl. The mobile phase was filtered through a millipore membrane filter paper and sonicated for 15 min for degassing prior to use.

Preparation of standard solutions:

Methanol and acetonitrile in the ratio of 50:50 (*v/v*) is used as diluent for the preparation of standard solutions. A standard stock solution of carisoprodol (1 mg/ml) was prepared by dissolving 100 mg of the drug in 100 ml diluent. Working standard solutions were prepared after the dilution of the stock solution with the same solvent. Five series of carisoprodol calibration solutions at the concentration values of 1, 3, 5, 10, 15, 20, 25 and 30 µg/ml were prepared from the stock standard solution by appropriate dilution with the diluent.

Preparation of stress degradation samples:

Stress degradation samples were prepared using different ICH recommended stress conditions such as acidic, alkali, oxidative and thermal[13].

Acid degradation

For acid degradation, 100 mg of carisoprodol was dissolved in 5 ml of 5N HCl in a 100 ml volumetric flask. The resulting solution was refluxed for 8 hours at 80°C on a heating mantle. After completion of the stress the solution was cooled and diluted to the volume with the diluent.

Alkali degradation

For alkali degradation, 100 mg of carisoprodol was dissolved in 5 ml of 5N NaOH in a 100 ml volumetric flask. The resulting solution was refluxed for 8 hours at 80°C on a heating mantle. After completion of the stress the solution was cooled and diluted to the volume with the diluent.

Oxidative degradation

Oxidative degradation was carried out at 80°C using 20% H_2O_2. To perform this, 100 mg of carisoprodol was dissolved in 5 ml of 20% H_2O_2 in a 100 ml volumetric flask. The resulting solution was refluxed for 8 hours at 80°C on a heating mantle. After completion of the stress the solution was cooled and diluted to the volume with the diluent.

Thermal degradation

Thermal degradation studies were performed in hot air oven at 105°C. For this study, 100 mg of carisoprodol powder was taken in glass petric dish and placed in oven at 105°C for 48 hrs. After specified time, the sample was cooled, transferred to a 100 ml volumetric flask and dissolved in 30 ml of diluent and made up to mark with the same solvent.

After degradation, all stress degraded samples were diluted to give a final concentration of 25µg/ml and filtered through a millipore membrane filter paper before injection in the chromatographic system.

General assay procedure:

Working standard solutions equivalent to 1 to 30 µg/ml carisoprodol were prepared by appropriate dilution of the stock standard solution (1 mg/ml) with the diluent. Prior to injection of the drug, the mobile phase was pumped for about 30 minutes to saturate the column thereby to get the base line corrected. 10 µl of each solution was injected automatically onto the column in triplicate and the peaks were determined at 240 nm. The peak areas of carisoprodol were plotted against the concentration to obtain calibration curve. The concentration of the carisoprodol was calculated either from the calibration curve or from the regression equation derived.

Assay of Carisoprodol in tablets

Ten tablets (Soma® CIV 250 mg tablet labeled to contain 250 mg/tablet, Meda Pharmaceuticals Inc. Somerset, New Jersey) were exactly weighed and powdered finely. An accurate amount of the powder equivalent to 100 mg of carisoprodol was transferred into a 100 ml volumetric flask and sonicated for 10 min with 50 ml of diluent. The flask was made up to volume with the same solvent. After filtration through millipore membrane filter paper, the solution was diluted with diluent to obtain a concentration of 25 µg/ml. Ten µl of the resulting solution was injected into the HPLC system. The drug amount was calculated by comparing the peak area with a standard solution at the same concentration value.

Results and discussion

Chromatographic conditions:

The main aim of the present investigation is to develop a stability indicating HPLC method with UV detector for the analysis of carisoprodol. Better peak shape and less retention time were observed by using a Zorbax Eclipse plus C18 (250 x 4.6 mm; 5 µm particle size) analytical column maintained at a temperature of 27±1 ^{o}C with a mobile phase consisting of 10 mM potassium dihydrogen orthophosphate, methanol and acetonitrile (60:20:20 *v/v/v*) at a flow rate of 0.7 ml/min. To get the maximum response of carisoprodol, 240 nm was selected as the detection wavelength which is the absorption maxima of carisoprodol. Under the optimized chromatographic conditions, the retention time of carisoprodol was about 2.907 min, which is very short (Figure 1).

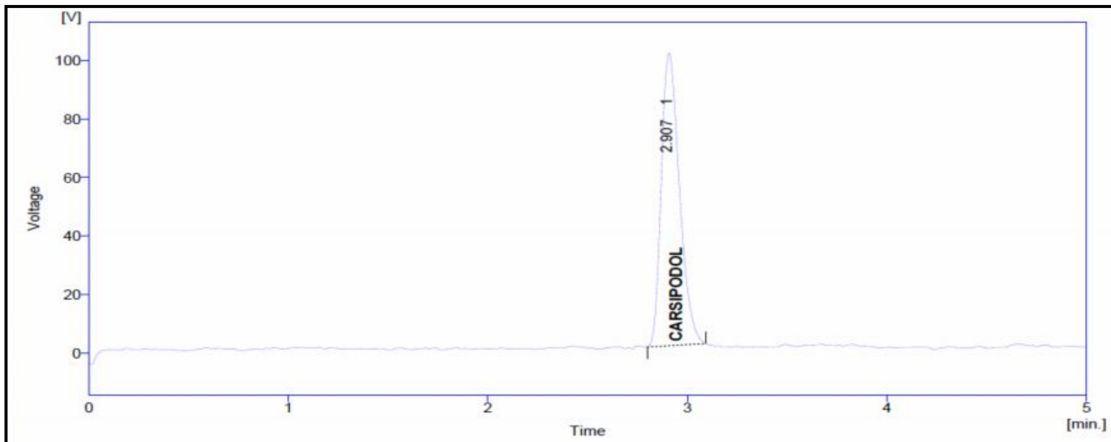

Figure 1: Chromatogram of carisoprodol under optimized chromatographic conditions

Validation of the method:

The developed method was successfully validated as per ICH guidelines by evaluating various parameters like system suitability, linearity, limit of detection, limit of quantitation, precision, accuracy, specificity, robustness and ruggedness[14].

System suitability:

To assess the system suitability of the method, six replicate analyses were performed at a concentration of 25µg/ml. The system suitability parameters were calculated and compared with the accepted criteria (Table 1). The values obtained demonstrated the suitability of the system for the analysis of the Carisoprodol.

Table 1: System suitability parameters

Parameters	Value	Recommended limits
Retention time	2.907	-
Peak area	1261.97 (%RSD – 0.10)	RSD ≤2
USP plate count	4681.833	> 2000
USP tailing factor	1.66	≤ 2

Linearity:

To evaluate the linearity of the proposed method, calibration curves were constructed by plotting the peak area of the calibration solutions in the range of 1– 30 µg/ml *vs* the carisoprodol concentration. The regression data of calibration curves are indicated in Table 2. The linearity of the method within the range of 1-30 µg/ml was satisfactory. The regression equation parameters like slope, intercept and correlation coefficient are acceptable.

Table 2: Linearity and regression analysis

Parameter	Value
Linearity (µg/ml)	1-30
Regresstion equation (y* = m x** + c)	y = 50.18 x + 3.852
Slope (m)	50.18
Intercept (c)	3.852
Correlation coefficient (R^2)	0.9995

*peak area

** Concentration of carisoprodol in µg/ml

Limit of detection and limit of quantitation:

The limit of detection (LOD) and limit of quantitation (LOQ) for carisoprodol was calculated using relative standard deviation of the response and slope of the calibration curve. The LOD and LOQ of a compound are defined as the lowest concentration that can be detected and lowest concentration of a compound that can be quantified with acceptable precision and accuracy, respectively. LOD and LOQ values for carisoprodol were found to be 0.092µg/ml and 0.299µg/ml. This indicates adequate sensitivity of the developed method.

System precision:

To assess the system precision, six replicate standard solutions (25µg/ml) of Carisoprodol were injected into the HPLC system. The percent relative standard deviation of peak responses was calculated. The results are summarized in Table 3. The value was within the acceptable criteria (<2.0).

Method precision:

Method precision was established by analyzing the tablet sample solution in six replicates. Percentage assay calculations were based on the calibration curve. Percentage relative standard deviation of the assay values were reported (Table 4). The value was within the acceptable criteria (<2.0).

Table 3: System and method precision

System precision		Method precision	
Concentration of drug (µg/ml)	**Peak area**	**Concentration of drug (µg/ml)**	**% Recovery**
25	1262.29	25	25.79
25	1261.58	25	25.45
25	1260.89	25	24.48
25	1262.99	25	25.36
25	1263.69	25	25.41
25	1260.37	25	24.25
Mean peak area - 1261.97		Mean recovery - 25.12	
SD – 1.26		SD – 0.038	
% RSD – 0.099		%RSD – 0.152	

Accuracy:

The accuracy of the developed method was validated by standard addition analysis. The preanalyzed tablet samples of concentration 10µg/ml were spiked with excess 80, 100, and 120% of standard carisoprodol. The total concentration of the carisoprodol was determined, to check for the recovery of the drug at different levels in tablet dosage form. The results (Table 4) meet the acceptance criterion (98-102%) for accuracy testing in the assay of pharmaceutical formulation.

Table 4: Accuracy of the method

Spiked level (%)	Concentration of carisoprodol (µg/ml)				% RSD	% Recovery
	Actual	**Spiked**	**found**	**Mean**		
80	10.00	8.02	18.15	18.10	2.83	100.54
80		8.02	17.56			
80		8.02	18.58			
100		8.02	20.35	20.09	2.80	100.43
100		10.00	20.47			
100		10.00	19.44			
120		12.04	22.69	22.42	1.31	101.92
120		12.04	22.47			
120		12.04	22.11			

Robustness

Robustness of the method was performed at a concentration of 10 ug/ml carisoprodol by making small deliberate changes in various chromatographic conditions like mobile phase composition, flow rate, column temperature and detection wave length. The percentage standard deviation of peak areas was calculated for each parameter and is presented in Table 5. The % RSD was found to be less than 1% indicating the robustness of the method.

Table 5: Robustness of the method

Parameter	Investigated range	Peak area	%RSD
Mobile phase ratio (*v/v/v*)	58:21:21	361.43	0.372
	60:20:20	359.47	
	62:19:19	362.01	
Column temperature (^{o}C)	25	360.98	0.086
	27	361.41	
	29	361.54	
Wavelength (nm)	238	360.44	0.296
	240	359.47	
	242	361.58	
Flow rate (ml/min)	0.6	362.33	0.305
	0.7	360.78	
	0.8	360.24	

Ruggedness

Ruggedness of the method was demonstrated at a concentration of 25 µg/ml carisoprodol by two different analysts, columns and system maintaining same experimental conditions. The results are given in Table 6. The % RSD was found to be less than 1% indicating the method was rugged.

Table 6: Ruggedness of the method

Parameter	Concentration of carisoprodol (µg/ml)		% Recovery	% RSD
	Taken	Found (n=6)		
Analyst I	25	25.19	100.76	0.264
Analyst II	25	25.45	101.80	0.362
Column I	25	24.88	99.52	0.519
Column II	25	25.36	101.44	0.336
System I	25	25.41	101.64	0.524
System II	25	24.95	99.80	0.624

Stability of standard and sample solution:

The stability of standard and tablet sample solutions of carisoprodol (30µg/ml) was determined by storing them at room temperature. The peak areas of the solutions were checked at 6, 12, 20, 26 and 30 hours of storage. The peak area of the stored solution was compared with freshly prepared sample. The results are shown in Table 7. The results indicated that the standard and tablet sample solutions of carisoprodol were stable for at least 30 hours at room temperature.

Table 7: Stability of standard and tablet sample solution

Standard Solution				
Time (hr)	**Peak area**			**% Difference**
0	1620.31	1610.11	1615.21	NA
6	1615.99	1520.31	1568.15	2.914
12	1622.47	1624.24	1623.36	-3.520
20	1621.88	1620.31	1621.10	0.139
26	1623.78	1621.69	1622.74	-0.101
30	1621.36	1621.47	1621.42	0.081
Tablet sample solution				
Initial	1622.32	1620.89	1621.61	NA
6	1621.89	1619.31	1620.60	0.062
12	1620.03	1621.45	1620.74	-0.009
20	1620.08	1622.20	1621.14	-0.025
26	1621.98	1621.06	1621.52	-0.023
30	1620.22	1620.31	1620.27	0.077

Stress degradation studies:

So as to assess the stability indicating property of the developed HPLC method stress degradation studies were carried out. Intentional degradation was done by exposing the tablet sample to the following stress conditions: acid (5 N HCl at 80 °C), alkali (5 N NaOH at 80 °C), oxidative (20% H_2O_2 at 80 °C) and thermal (105 °C). The ability of the developed method to determine the carisoprodol response in the presence of its degradants was studied. In the stress degradation studies, carisoprodol was found to degrade under oxidative stress conditions employed. However it was found to be stable to the acid, alkali and thermal degradation conditions employed. Except the oxidative stress, in all the remaining stress conditions only a small percentage of degradation was observed. The results of forced degradation studies are included in Table 8. Chromatograms obtained for carisoprodol under different stress conditions are shown in Figures 2-5. The developed HPLC method could resolve the drug from their degradants which prove the stability indicating power of the developed method.

Table 8: Results of stress degradation studies

Type of stress	Peak area	% Recovered	% Degraded
Undegraded	1262.29	100.00	-
Acid	1261.58	99.94	0.06
Alkali	1260.77	99.88	0.12
Oxidative	361.01	28.60	71.40
Thermal	1260.47	99.85	0.15

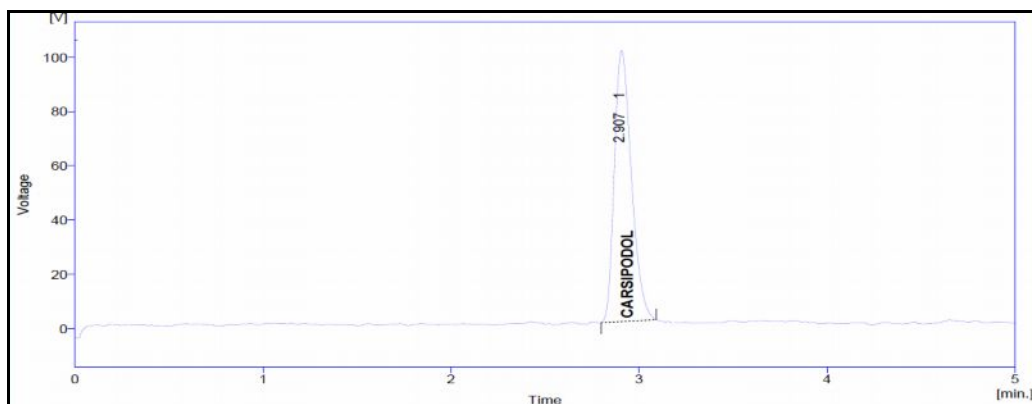

Figure 2: Chromatogram of 5N HCl carisoprodol degradant

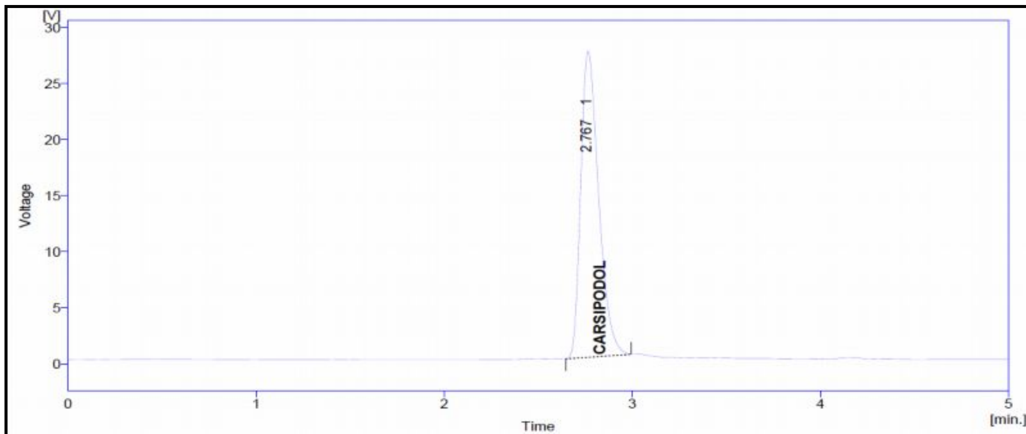

Figure 3: Chromatogram of 5N NaOH carisoprodol degradant

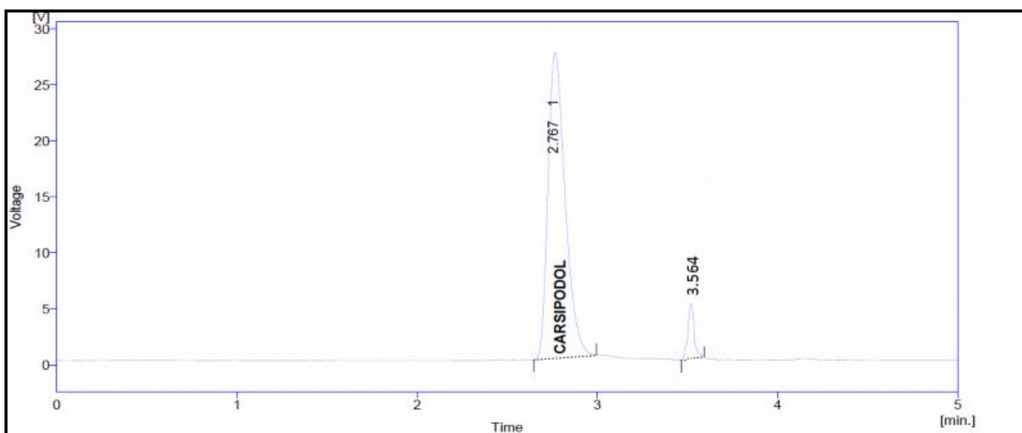

Figure 4: Chromatogram of 20% H_2O_2 carisoprodol degradant

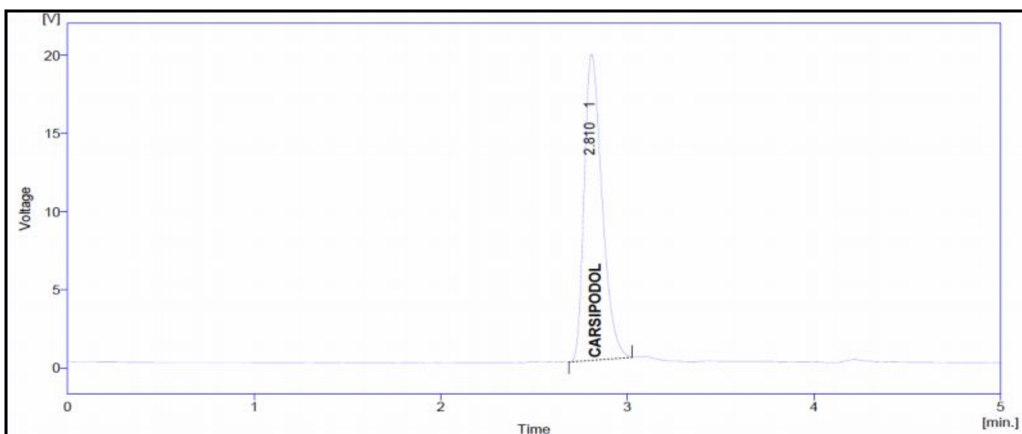

Figure 5: Chromatogram of dry heat carisoprodol degradant

Analysis of a tablet dosage form:

The developed stability indicating HPLC method was applied for the assay of carsiprodol in commercially available tablets. Experimental results of the amount of carsiprodol in tablets were expressed as a percentage of label claim (Table 9). The results are in good agreement with the label claims thus suggesting that there is no interference from any of the excipients that are present in tablets. From these results, it is proved that the proposed method can be applicable for the analysis of carsiprodol in tablet dosage form with satisfactory level of selectivity, accuracy, and precision.

Table 9: Analysis of carisoprodol in tablets

Labeled claim (mg)	Found (mg)	Mean	% Recovery	% RSD
250	251.26	250.22	100.088	0.243
250	249.85			
250	249.56			

Conclusion

The developed HPLC method was found to be simple, sensitive and apt for the determination of carisoprodol in the presence of its stress degradation products. The results of validation parameters proved that the method is precise, accurate, robust, rugged and specific for the analysis of carisoprodol. Furthermore, the method is less time consuming. Therefore, it may be advantageous for routine analysis of carsiprodol in quality control laboratories. Thus, the proposed HPCL method can represent an alternative for the already existing HPLC method[12].

Acknowledgements

The authors are thankful to authorities of Acharya Nagarjuna University, Guntur and Chalapathi College of Pharmacy, Guntur for providing facilities to carry out the present work and greatly acknowledge to carisoprodol was a gift sample from Arabindo Laboratories Pvt.Ltd, Hyderabad.

References:

1. Bramness JG, Furu K, Engeland A. Carisoprodol use and abuse in Norway.A pharmacoepidemiological study. Braz J Clin Pharmacol., 2007, 64; 210–218.
2. Peter ET, Jason U. Commonly used muscle relaxant therapies for acute low back pain: a review of carisoprodol, cyclobenzaprine hydrochloride, and metaxalone. Clin Ther., 2004, 26; 1355–1367.
3. Lee R, Michele L, William W, Harry S. Double-blind, placebo-controlled trial of carisoprodol 250-mg tablets in the treatment of acute lower-back spasm. Curr Med Res Opin., 2008, 24; 551-558.
4. The United States Pharmacopoeia, United States Pharmacopeial Convention Inc., Rockville, MD, USA, 2004, p. 340.
5. Angela SM, Samuel RG. Identification and determination of carisoprodol in tablets by liquid chromatography/mass spectrometry. Microgram J., 2004, 2; 36-41.
6. Skinner D, Mckemie S, Stanley. Quantitative determination of carisoprodol and its metabolites in equine urine and serum by Liquid chromatography-tandam mass spectrometry, Chromatographia, 2004, 59 (Supplement 1); S61-S67.
7. Kucharczyk N, Segelman FH, Kelton E, Summers J, Sophia RD, Mahrous H, Heath E. Gas chromatographic determination of carisoprodol in human plasma. J Chromatogr Biomed Appl., 1986, 377; 384-390.
8. Kintz P, Marigin P, Lugnier AAJ, Chaumont AJ. A Rapid and sensitive gas chromatographic analysis of meprobamate or carisoprodol in urine and plasma. J Anal Toxicol., 1988, 12; 73-74.
9. Fisher J, Sherma J. Analysis of carisoprodol tablets by HPTLC with visible absorbance densitometry. Acta Chromatogr., 2001, 11; 96-101.
10. Guohong W, Kim H, Rekha B, Warren R, Christine M, Cynthia C, Michael V, James S. Validation of a new homogeneous immunoassay for the detection of carisoprodol in urine. J Anal Toxicol., 2011, 35; 108-112.
11. Ravi M, Veeraiah T, Reddy ChVR. Spectrophotometric determination of carisoprodol in pure and pharmaceutical forms using triphenyl methane dyes. Int J Pharma Sci Res., 2015, 6; 2936-2943.
12. Rohith T, Ananda S, Netkal MMG. Method development and validation of carisoprodol and its impurities by ultra violet-high performance liquid chromatography. Adv Anal Chem., 2013, 3; 15-19.

13. ICH Validation of analytical procedures; Text and methodology; Q2(R1), International Conference on Harmonization, 2005.
14. ICH Stability Testing of New Drug Substances and Products, Q1A(R2), International Conference on Harmonization, 2003.

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