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Development of a new HPTLC Method for the Estimation of Carisoprodol and its Standardization by Comparison with A Reported HPLC Method.

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ABSTRACT

An analytical method for the estimation of Carisoprodol in bulk drug and in pharmaceutical dosage forms by HPTLC is developed. The method is simple, fast and accurate and can be used for routine analysis of commercial Carisoprodol tablets. The developed method uses pre-coated silica gel $60F_{254}$ as stationary phase. The mobile phase used was a mixture of Chloroform: Acetone (80:20 %v/v). The RF value was 0.65.The detection of spots were as carried out at 366 nm after derivatization with the derivatizing agents, Antimony (III) chloride and Furfuraldehyde. The calibration curve was found to be linear between 50 and 300µg/spot with a correlation coefficient of 0.99895. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 25µg/spot and 50 µg/spot respectively. The proposed method has been applied successfully for the determination of Carisoprodol in pharmaceutical dosage forms. No significant interference was observed from excipients, coloring and flavoring agents commonly used in the formulation. The mean recovery of drug from tablets was found to be in the range of 102.2% to 104.8%.

Keywords: carisoprodol, estimation, detection, tablets.

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INTRODUCTION

Carisoprodol works by blocking pain sensations between the nerves and the brain. Chemically, carisoprodol is [2-(carbamoyloxymethyl)-2-methylpentyl]N-propan-2-ylcarbamate [1]. It is used to relax muscles and help to alleviate the pain from muscle spasms [2]. The structure of Carisoprodol is such that it does not have any UV chromophore with significant absorbance. Therefore, the USP assay method for Carisoprodol tablets employs a liquid chromatography equipped with a refractive index detector [3].The literature survey revealed that several methods were reported based on titrimetric analysis, infrared spectroscopy, nuclear magnetic resonance (NMR),gas chromatography, liquid chromatography (LC) and gas chromatography-mass spectrometry (GC-MS) for the estimation of Carisoprodol [4-12].The methods reported are sophisticated, but costly and time consuming. The proposed HPTLC method has the advantage that the substance placed on the plate will remain there avoiding detection problems associated with non- elution and de-masking by solvent front. The applications in HPTLC have been greatly speeded up by the use of automated sample application device and accurate densitometers.

MATERIALS AND METHODS

Chemicals and Reagents

Carisoprodol working standard was procured as gift sample from Allied Chemicals & Pharmaceuticals Pvt.Ltd.Silica gel 60F $_{254}$ TLC plates (E.Merck, Mumbai) were used as the stationary phase. Carisoprodol tablet was purchased from the local market (Carisoma 350mg, contain Carisoprodol 175mg). A Camag HPTLC system comprising of CamagLinnomat-IV automatic sample applicator, Hamilton syringe, Camag TLC Scanner 3, Camag Win CATS software, Camag twin-trough chamber and ultra sonicator were used during the study.

Preparation of working standard solutions

Working standard of Carisoprodol (50mg) was weighed accurately and dissolved in 30 ml of methanol and the volume was made up in a 50ml volumetric flask with the same solvent. This stock solution had a concentration of 1000mcg/ml Carisoprodol. From the above stock solution the final solution of concentration 200µg/ml was prepared and this solution was used for application on HPTLC plate.

Preparation of sample solution

The contents of 20 Carisoma tablets were ground to fine powder. Weight equivalent to 50 mg each of Carisoprodol was transferred to a 50ml conical flasks and dissolved in 30ml of methanol and mixed gently. This solution was transferred into a 50ml volumetric flask through a whatmann filter paper n .40.The residue in the conical flask was extracted with further 10 ml of methanol and that was passed through the same filter paper and the final volume in each case was made up to 50 ml with 4:1 chloroform: acetone mixture. From the above stock solution the final solution of concentration of 5mcg/ml or 5ng/µl was prepared.

Chromatographic conditions -Sample application

The chromatographic estimation was performed using stationary phase, pre coated silica gel 60F $_{254}$aluminium sheets (20 × 10 cm, pre washed and dried in an oven at 50° for 5 min). Linear ascending development was carried out in 10 x 10 cm twin trough glass chamber equilibrated with mobile phase. The optimized chamber saturation time for mobile phase was 20minutes at 25±2°C with a relative humidity of 60±5%. 10ml of the mobile phase (5 ml in trough containing the plate and 5 ml in other trough) was used for the development and allowed to migrate a distance of 85 mm from the point of sample application.

Selection of mobile phase

Several systems were tried by trial and error method. Chloroform: acetone mixture (80:20) were proved to be the good solvent system and gave RF value of 0.67 (±0.05). The TLC plates were developed with Antimony (III) chloride/Furfuraldehyde reagents.

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Documentation

The various conditions for documentation were selected. After development, the plates were photographed in various conditions under UV 254 nm and UV 366 nm after derivatization.

Development of calibration curve

The programmer was set with a band width of 6mm and flow rate of 4 µl / second. Standard solution was prepared by dissolving 50 mg Carisoprodol in 10 ml methanol and 10 µL ,20 µL, 30 µL, 40 µL, 50 µL, 60 µL, were applied on the same plate. Following chromatography and densitometry, the plate was documented. The calibration curve was prepared by plotting peak area versus concentration (µg) .The method was validated by establishing linearity, accuracy, inter-day and intra-day precision, specificity, repeatability of measurement of peak, as well as repeatability of sample application. The limit of detection and limit of quantification were also determined. Figure.1 shows the densitogram of Carisoprodol 200 µg. The peak areas were noted. The data and the linearity plot obtained are shown in Table.1 and Figure.2 respectively.

Table 1: The linearity data of Carisoprodol standard solutions

Track no.	Concentration in µg	Peak Area
1.	50	656.26
2.	100	1271.19
3.	150	1684.89
4.	200	2225.03
5.	250	2825.10
6.	300	3358.69

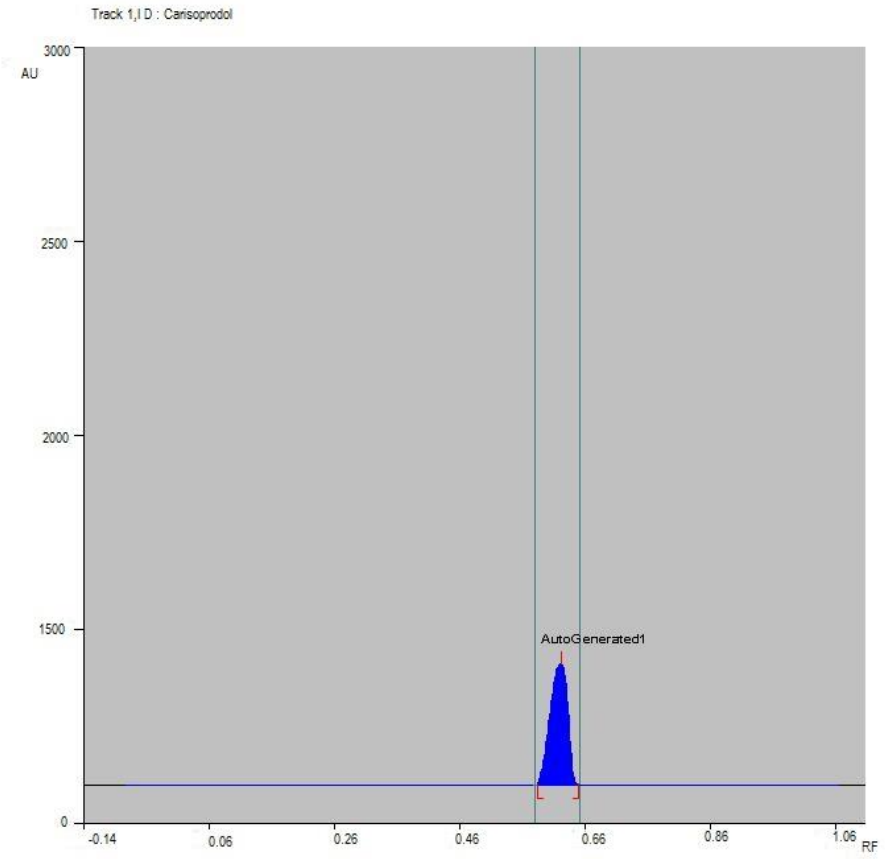

Figure 1: Densitogram of Carisoprodol 100µg

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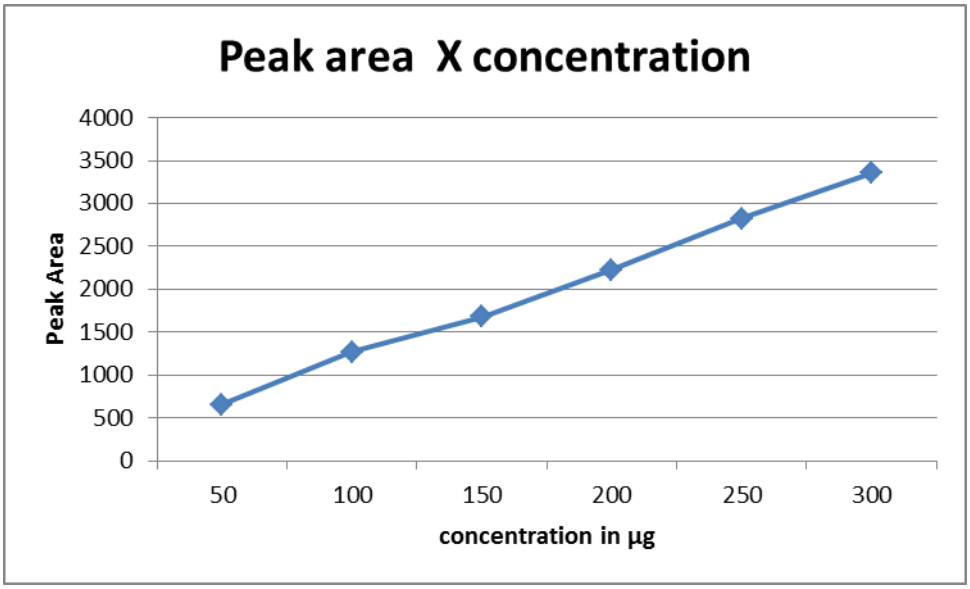

Figure 2: Linearity plot of Carisoprodol standard solutions

Assay procedure for tablet solution

For the analysis of the marketed formulations, 10 µl of extracted solution of the marketed formulation was spotted on to the same plate to get a concentration of 200µg, followed by development and scanning. The content of the drug from Carisoma tablet was calculated from the peak area recorded and the data obtained is shown in table.2.

Table 2: The assay results of Carisoprodol standard & Carisoma tablet

Track	Sample	Area	Label claim	%Label claim
1.	Carisoprodol (Standard)	2236.5 AU	177.02mg	101.14%
2.	Carisoma tablet	2231.6 AU		

METHOD VALIDATION

The method was validated as per ICH guidelines [13] with respect to linearity, range, accuracy, specificity, precision, limit of detection, limit of quantitation and robustness.

Linearity and Range

Areas under curve (AUC) of five standard mixtures of different concentrations were determined and a calibration plot was obtained by plotting AUC area verses concentration equation. Linearity range was found to be in the range of 50 to 300µg/spot with a correlation coefficient of 0.99895. The LOD and LOQ were found to be 25 µg/spot and 50 µg/spot respectively.

Precision (Repeatability)

Precision of the method was tested by performing intra-day and inter-day studies. Intra-day precision was determined by analyzing standard solutions of Carisoprodolin three times on the same day, and inter-day precision was determined by analysis of the same standards on three different days over a period of one week. The intra-day and inter-day relative standard deviations were in the ranges 0.84–1.06% and 1.19–1.36%.These low values indicated that the method is precise.

Accuracy

The recovery study of the drug was carried out for accuracy parameters at multiple levels. Two sample weights of Carisoprodol marketed tablets equivalent to 10 mg was accurately weighed out and transferred to two 100 ml standard flask, added10 mg of Carisoprodol R.S to each flask and extracted by adding 20ml mobile phase initially, the volume was finally made up to 100 ml with mobile phase. Dilutions were made to get a concentration of 100µg/ml and recovery studies were performed. Percentage recovery was found to be within limits, as listed in Table.3

Table 3: Data of recovery study

Sample	Volume used	Amount recovered	%Recovery	% RSD
Carisoprodol 10mg + Carisoma tablet 10mg	30µl	10.22	102.2	1.12
	50µl	10.48	104.8	1.06

Specificity

No interference with the excipients in tablet was observed during analysis. Hence the method was found to be specific.

Robustness

The robustness of the method was studied for three different amounts (100, 150, and250µg per band) of Carisoprodol by determining the effects of volume of mobile phase used (±0.5%), time from sample application to chromatography (±20 min), and time between chromatography and scanning (±20 min). The RSD (%) of peak area was calculated for each change of conditions and found to be within the range stipulated by ICH guidelines (Table.4).

Table 4: Result of robustness testing of proposed method

Condition	RSD % (n=3)
volume of mobile phase (±0.5%)	1.01
time from sample application to chromatography (±20 min)	1.14
time between chromatography and scanning (±20 min)	1.13

Ruggedness

In intra-day, inter-day variation and the result of estimation by proposed method were found to be satisfactory which indicates ruggedness of the method.

RESULTS AND DISCUSSION

Carisoprodol is not freely soluble in water and hence methanol was used as solvent. Tablet powder was extracted with this solvent. A variety of mobile phases were used for separation of Carisoprodol from other excipients present in formulations. The mixture chloroform: acetone (80:20) enabled satisfactory resolution with good peak shape and RF values of 0.67 ± 0.05 (Figure.1.) The method was validated in accordance with ICH guidelines .The method was linear in the range 50–300µg per band of Carisoprodol (with correlation coefficient 0.9985, n = 6). The accuracy of the analysis was evaluated by the determination of recovery at three different concentrations of the drug in the dosage form. The results indicated that the method enables accurate estimation of the drugs in the tablet dosage form .The assay value for the marketed formulations were found to be within the limits, as listed in Table.2. The low RSD value indicated the suitability of the method for routine analysis of Carisoprodol in pharmaceutical dosage forms.

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COMPARISON OF THE PROPOSED METHOD WITH OFFICIAL METHOD

The proposed method was compared with the HPLC method and the data are given Table .5

Table 5: Comparison of HPTLC method with HPLC method

Carisoma	Amount estimated by		% of Label claim	
	HPLC method	Proposed method	HPLC method	Proposed method
	176.02	177.02	100.58%	101.02%

HPLC Method

The official method of estimation of Carisoprodol in dosage forms is the HPLC method. Hence comparison is done with HPLC method using the same mobile phase and same flow rate for analysis of dosage forms.

ANALYSIS OF CARISOMA TABLETS BY HPLC METHOD

Reagents used: Acetonitrile, orthophosphoric acid and pH was adjusted to 3.1 with trimethylamine.(The solvents used were HPLC grade supplied by Emerck Ltd.)

Preparation of Reagents

A 30:70 mixture of acetonitrile & dilute phosphoric acid was prepared, filtered and degassed for use.

Preparation of Standard

Accurately weighed 50mg of Carisoprodol R.S. was dissolved in diluting solution in a volumetric flask and made up the volume to 100ml with mobile phase to get a concentration of 200 µg/ ml of Carisoprodol.

Preparation of Sample

10 tablets were weighed and powdered. Weight of the powder equivalent to 30mg Simvastatin was accurately weighed and transferred to a 100ml conical flask and added 20ml of diluting solution; mixed well and filtered through a Whatman No:1 filter paper. Extracted the residue with 20ml of diluting solution and filtered through the same filter paper. The residue on the filter paper was washed with diluting solution. Finally transferred to a 100ml volumetric flask and the volume was made up to 100ml with mobile phase to get a concentration of 200 µg/ ml.

Details of Analysis

Mobile phase	:	Acetonitrile / dilute phosphoric acid
Ratio	:	30:70
Flow rate	:	1ml/mt
Column	:	Lichrosorb RP – 18
Detector	:	UV detector
Wavelength	:	232.2nm
Sample	:	Carisoma tablet

After initial stabilization 25µl each of standard and samples were injected separately into the column. The pump was adjusted to flow rate of 1ml/minute and detector was adjusted to 238nm wavelength. The chromatograms obtained were recorded. Concentration of the drug in the tablets were calculated from the peak area of the sample with that of standard, according to the following formula.

CONCLUSION

Assay of Carisoprodol in marketed formulations were carried out and compared with the official HPLC method. The results obtained are comparable and no interference from excipients were observed. Hence it can be concluded that the developed HPTLC technique is simple, fast, precise, specific and the validation proved that the method is reproducible and selective for the analysis of Carisoprodol in bulk drug and in tablet formulations.

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