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Short communication

FTIR assay method for UV inactive drug carisoprodol and identification of degradants by RP-HPLC and ESI-MS

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ABSTRACT

A new method of analysis has been developed for UV inactive drug carisoprodol using FTIR spectroscopy. These methods were validated for various parameters according to ICH guidelines. The proposed method has also been successfully applied for the determination of the drug concentration in a tablet formulation. The method proved to be accurate (mean percentage recovery between 95 and 105%), precise and reproducible (relative standard deviation < 2%), while being simple, economical and less time consuming than other methods and can be used for routine estimation of carisoprodol in the pharmaceutical industry. The developed method also implicates its utility for other UV inactive substances. The stability of the drug under various stress conditions was studied and the drug was found to be particularly susceptible to alkaline hydrolysis. Degradation products of the alkaline hydrolysis were detected by RP-HPLC and tentatively identified by ESI–MS.

1. Introduction

Carisoprodol, (*RS*)-2-{[(aminocarbonyl)oxy]methyl}-2-methylpentyl isopropylcarbamate, (Fig. 1), is a commonly prescribed muscle relaxant and analgesic. However, the patients are at risk for dependence, toxicity and withdrawal which are associated with its active metabolite meprobamate [1]. Therefore, carisoprodol requires a precise method of analysis in various pharmaceutical formulations.

Carisoprodol is an UV inactive substance due to absence of appropriate functional groups to absorb UV or visible lights. Chemical derivatization using chromogenic reagents is also difficulty for this drug due to lack of suitable functional group. For this reason, a titrimetric method of analysis for carisoprodol is described in various pharmacopoeias [2–4]. Various HPLC assay methods have been developed for the estimation of carisoprodol in pharmaceutical dosage forms [5–7]. LC–MS methods have also been reported for simultaneous determination of carisoprodol along with other components in pharmaceutical formulations [8,9]. These methods suffer from the disadvantage of injecting high drug concentration (>2500 ppm) to the column to obtain the chromatogram leading to column inactivation.

In recent years, MIR (mid infrared) spectroscopy has emerged as a rapid, simple, precise, accurate and economical analytical method for the quantitative estimation of pharmaceuticals in the presence of excipients [10,11]. Characteristic strong vibrational bands of the functional groups in the MIR spectra are more useful than low intensity absorption bands of the NIR (near infrared) to distinguish and estimate closely related derivatives [10]. Hence, considering all information about the analysis of carisoprodol, the present study describes a FTIR method for the quantitative analysis of this drug in bulk and tablet dosage forms. The method was validated and compared as per ICH guidelines [12]. The ICH guideline also emphasizes on stress testing of pharmaceuticals to identify the possible degradation products in order to determine the inherent stability of the molecule as well as establishing the degradation pattern [13]. Therefore, forced degradation studies of carisoprodol have also been performed to identify the possible degradants employing RP-HPLC and ESI–MS.

2. Material and methods

2.1. FTIR method development

Carisoprodol was obtained as a gift sample from M/s Harman Finochem Ltd. IR grade KBr and solvents of HPLC grade were purchased from Merck, Mumbai. A marketed formulation (Carisoma compound, Wallace Ltd, Batch No- CCR/3004) was obtained from a local drug store. The infrared spectra of the solid samples were

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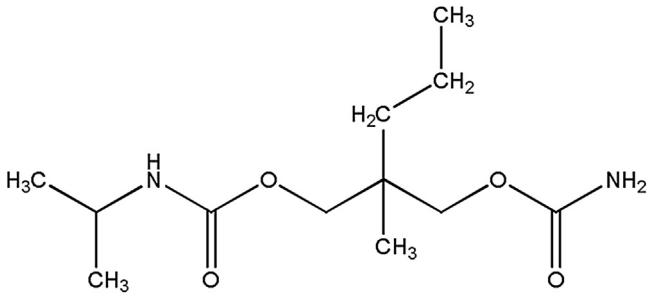

Fig. 1. Structure of carisoprodol.

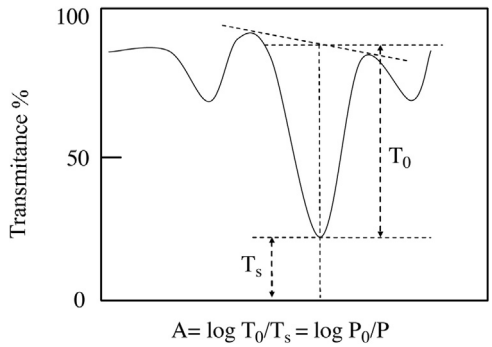

Fig. 2. the baseline method for determination of the absorbance of an absorption maximum.

recorded over a spectral region of 4000–400 cm^{-1} using a Perkin Elmer Spectrum RX1 FTIR spectrophotometer. The pellets were prepared applying 5 ton force using the hydraulic press each time. Spectra were recorded with 4 cm^{-1} resolution and 4 scans per sample in order to get a good signal to noise ratio and reproducible spectrum. These conditions were kept constant throughout the experiment. All the spectra were obtained in transmittance mode.

The baseline technique was used for the quantitative analysis of the drug samples as shown in Fig. 2 [10,14]. The values of P_0 (amount of light incident on the sample) and P (amount of light transmitted by the sample) were calculated using the infrared spectrum of the samples. The quantitative analysis was carried out using the regression method.

2.1.1. Preparation of stock solution

Accurately weighed 40 mg of carisoprodol was transferred to a 20 mL volumetric flask, dissolved in anhydrous chloroform and the volume was made up to the mark to obtain a sample of final strength 2 mg/mL.

2.1.2. Preparation of samples

Five different concentrations of 0.4%, 0.6%, 0.8%, 1.0%, 1.2% w/w were prepared by adding 0.4, 0.6, 0.8, 1.0, 1.2 mL of carisoprodol stock solution to 200 mg of KBr in a glazed mortar mixed properly with the help of pestle, dried in hot air oven for 15 min at 60 °C to produce a homogenous powder. Pellets weighing 70 mg were prepared from this powder, containing 0.4, 0.6, 0.8, 1.0, 1.2% w/w of carisoprodol, respectively. The FTIR spectra were recorded and the standard curve was plotted by taking absorbance values of carisoprodol, calculated using baseline technique, versus concentration.

2.2. Method validation

2.2.1. Linearity

Linearity of the method was established by taking five independent concentrations (0.4–1.2% w/w) of the carisoprodol and the data analysis was performed using least square regression analysis.

2.2.2. Accuracy

Different levels of drug concentrations were prepared from independent stock solution and analyzed ($n = 9$) to access the recovery study in terms of the percentage relative standard deviation (%RSD) and mean percentage recovery. Standard addition method was also performed to give additional support to accuracy of the developed assay method. Different concentrations of pure drug (1.5, 1.6 and 1.7 mg/mL in chloroform) were added to a known preanalysed formulation sample and the total concentration was determined using the proposed methods ($n = 3$).

The percentage recovery of the added pure drug was calculated as,

$$\%\text{Recovery} = \frac{(\text{Cv} - \text{Cu}) \times 100}{\text{Ca}}$$

Where, Cv is the total drug concentration measured after standard addition.

Cu is the drug concentration in the formulation.

Ca is the drug concentration added to formulation.

2.2.3. Precision

Repeatability was studied by analyzing different levels of drug concentrations (n = 9) prepared in triplicates from an independent stock solution. The sample was analyzed by using 0.8%, 1.0%, 1.2% w/w dilutions with KBr powder. The % RSD of the predicted concentrations i.e. 80%, 100%, 120% from the regression equation was taken as precision.

2.2.4. Limit of detection and limit of quantitation

The LOD and LOQ of carisoprodol by the proposed method were calculated as 3.3 σ/S and 10 σ/S, respectively where S is the slope of the calibration curve and σ is the standard deviation of the y-intercept of the regression equation.

2.2.5. Robustness

Robustness of the proposed method was determined by deliberately varying the weight of the carisoprodol-KBr mixture by ± 2 mg. Three different concentrations (LQC, MQC and HQC) were prepared and robustness was determined as %RSD.

2.2.6. Estimation in tablets

Ten tablets containing carisoprodol were weighed and powdered from which a portion equivalent to 40 mg of carisoprodol was accurately weighed and extracted with 10 mL of chloroform with the help of a sonicator. The extract was filtered hot using a Hirsch funnel of 5 mm diameter into a 20 mL volumetric flask. The containers and filtration assembly were rinsed with 5–6 mL of warm anhydrous chloroform to facilitate complete extraction of carisoprodol. The volumetric flask was cooled to room temperature and made up to volume with anhydrous chloroform to provide a solution equivalent to 2 mg/mL. From this stock solution, 0.8 mL was pipetted out and poured into a 200 mg KBr in a mortar, mixed properly and dried at 60 °C for 15 min in a hot air oven. Three homogenous pellets of 70 mg each were prepared and employed for quantitative estimation using FTIR.

The FTIR assay method for carisoprodol was also compared to the USP method using HPLC [5]. For this purpose, tablet powder equivalent of 76 mg of carisoprodol was weighed, dissolved in