DETERMINATION OF BENZALDEHYDE GENOTOXIC IMPURITY IN LACOSAMIDE DRUG SUBSTANCES USING HPLC TECHNIQUE

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ABSTRACT

Benzaldehyde has been highlighted as one of the potential genotoxic impurities (PGIs) in Lacosamide drug substances. A sensitive HPLC method was developed and validated for the determination of benzaldehyde in lacosamide drug substances. HPLC method on Kromasil C18 column (250 X 4.6 mm, 5µm), with UV detection at 250 nm was used. The proposed method was cost effective, specific, linear, accurate, rugged and precise. The calibration curves showed good linearity over the concentration range of 1.13 μg/g to 5.64 μg/g with respect to the sample and the correlation coefficient was 0.9981. Accuracy results were well in the range 96.28 to 101.41%. and method very sensitive.

KEYWORDS: Impurity, HPLC, TTC, ICH guidelines, Validation.

INTRODUCTION

Lacosamide (LAC) is chemically [R]-2-acetamido-N-benzyl-3-methoxypropionamide. The drug is a functionalized D-Serine derivative in the R-configuration. Its molecular formula C_{13}H_{18}N_{2}O_{3}, the formula weight is 250.301. LAC acts by the mechanism of the enhancement of slow inactivation of voltage gated sodium channel. LAC is an anti-epileptic drug is used to treatment of partial-onset seizures in people with epilepsy 1. LAC is available in 50, 100, 150 and 200 mg tablet dosage form either individually or combination with other drugs, some of the brands include Ictrol, Lacasa, Lacopsy, Lacosam, Seizgard, Seizlac and Vimpat3,4.

Benzylamine was used as a reagent in the amidation step of LAC synthesis5,6. Benzaldehyde was generated as byproduct during this step and identified as a genotoxic impurity according
to the guideline\cite{7,8}. The chemical structures of Lacosamide and Benzaldehyde are presented in fig. 1.

\begin{figure}[h]
\centering
\includegraphics[width=0.7\textwidth]{structure.png}
\caption{Chemical structure of (a) Lacosamide (b) Benzaldehyde}
\end{figure}

Detailed literature survey reveals that the many analytical methods are available for the determination of the LAC drug individually or in combination with other drugs\cite{9-15}. To our knowledge no HPLC method available for quantitative determination of benzaldehyde in LAC drug. In this paper, we report a validated HPLC method for quantitative determination of benzaldehyde in LAC drug.

**MATERIALS AND METHOD**

**Chemical and Reagents**

HPLC grade of water, orthophosphoric acid, methanol and acetonitrile was purchased from Merck, Mumbai India. The Benzaldehyde standard (Purity 99.0%) was from sigma alderic. All pure drug substances are used for research purpose procured jubilant life sciences.

**Instrumentation and Chromatographic Conditions**

HPLC system (Agilent Technologies 1200 series) equipped with quaternary pump, sampler, column heater and UV-visible variable wavelength detector (1200 VWD) was employed for analysis. Analysis of solution was carried out at 245 nm with Kromasil C18 reversed-phase analytical column (250 X 4.6 mm, 5µm). The isocratic mobile phase consisted a mixture of buffer (0.1% orthophosphoric acid) and acetonitrile in the ratio of 60:40 (v/v) throughout the analysis. The mobile phase was degassed by vacuum filtration before use. The flow rate of the mobile phase was 1.0 mL/min and total run time was 15 min. The column temperature was controlled at 40°C and injection volume was 10 µL.
**Preparation of standard and sample solutions**

A stock solution of benzaldehyde impurity was prepared by dissolving the appropriate amount of benzaldehyde impurity in diluents. Working concentration of 0.1 μg/mL impurity solution were prepared from the stock solution and used as standard solution. The sample solution (26600μg/mL) were prepared by weighing 532 mg of drug substances and transferred to 20 mL volumetric flask. Filter the solution through 0.45μ nylon filter and used the clear solution for injection.

**RESULTS AND DISCUSSIONS**

The evaluation limit for LAC genotoxic impurity in the selected drug has been calculated based on TTC and a maximum daily dose of drug i.e. 400 mg. A maximum daily exposure target of genotoxic impurities is 1.5 μg per day per person.[7]

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\text{Evaluation Limits (µg/g)} = \frac{1.5 \, \mu g \, / \, \text{day}}{\text{dailydose (g/day)}} = \frac{1.5}{0.400} = 3.75 \, \mu g \, / \, g
\]

Hence the limit for LAC impurity is 1.5 / 0.400 = 3.75 μg/g

The desired specificity of the method was achieved on Kromasil C18 (250 x 4.6 mm, 5µm) column with buffer (0.1% Orthophosphoric acid) and acetonitrile in the ratio (60:40, v/v) as mobile phase. Impurities were monitored by using detector at 250 nm (fig. 2). Flow rate was 1.0 mL/min and the column oven temperature was 40 °C. The injection volume was 30 µL and total run time of the method was 15 min. mobile phase was used as diluent.

![Fig.2: Absorption spectrum of Benzaldehyde](image)

In order to validate the developed HPLC method, Validation characteristics such as specificity, detection limit, quantitation limit, linearity, precision, accuracy robustness and
solution stability were considered as per ICH guideline\textsuperscript{16}. Specificity of the method for the estimation of the benzaldehyde impurity in drug described in the present study was proven by injecting separately solutions of blank, standard, sample and sample spiked with benzaldehyde standard individually. Figure 3 shows overlays of chromatograms and prove that the no interference around the retention time of benzaldehyde; also the base line did not show any significant noise around the peak. The retention times of LAC and benzaldehyde have recorded 3.8 and 8.6 min, respectively.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{overlap_chromatogram.png}
\caption{Overlap chromatogram of (1) blank, (2) sample, (3) spiked sample}
\end{figure}

Sensitivity was determined by establishing limit of detection (LOD) and limit of quantification (LOQ) through signal-to- noise ratio of 3:1 and 10:1 respectively, by injecting a series of dilute solution having a known concentration. LOD of the impurity is defined as the lowest concentration that can be detected and LOQ is the lowest concentration that can be quantified with acceptable precision and accuracy. The LOD and LOQ value for the impurity were found to be 0.37 µg/g and 1.13 µg/g. Precision study was carried out at LOQ by injecting six individual preparations of impurity and calculating % RSD i.e. 1.52 %. The representative chromatograms were showed in figure 4.
The method was observed good linearity (Fig. 5) in the 250 nm absorption with increasing concentration of benzaldehyde standard LOQ to 150 % of the evaluation limit. The result shows that an excellent correlation ($R^2$) =0.9981< 0.990 existed between the peak area and the concentration of the impurity over the entire concentration.

Accuracy and precision were validated on a LAC sample spiked with benzaldehyde at three concentration levels covering the specified range with six replication for 3.75 µg/g benzaldehyde concentration and three replicates for 1.88 and 5.63 µg/g. The individual percent recoveries for all preparation were from 96.28-101.41 % which is well within
acceptance criteria 80 % to 120 % and the % RSD of six replicate at, 3.75 µg/g was 1.12 < 5.0. Solution stability on the standard concentrations was tested for 2 h, 4 h, 12 h and 24 h time point at laboratory temperature. Solution stability runs indicated that the benzaldehyde is stable up to 24 h at observed room temperature. To determine the robustness of the method the experimental conditions were deliberately altered and the system suitability result was evaluated. To study the effect of flow rate, it was changed by 0.2 units from 1.0 mL/min to 0.8 mL/min and 1.2 mL/min. The effect of column temperature was studied by changed 5 °C units from 40 °C to 35 °C and 45 °C. The results were found that the deliberate changes in the method i.e. flow rate of mobile phase and column oven temperature has no impact on system suitability. The three manufacturing batches of LAC drug substance were analyzed by using validated method for determination of impurity and found the benzaldehyde was not detected in all three batches. The overlay chromatogram was presented in fig. 6.

Fig. 6: Overlap chromatogram of three manufacturing batches of lacosamide sample with standard solution

CONCLUSION

The isocratic HPLC method developed for the quantitative determination of genotoxic benzaldehyde impurity in LAC drug substances is linear, precise, accurate, rugged and robust. Satisfactory results were obtained from validation of the method as per ICH guideline. This method exhibited an excellent performance in terms of sensitivity and specificity with no sample matrix and impurity interference observed and can be used for routine analysis to determine the content of benzaldehyde.
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