DETERMINATION OF GENOTOXIC IMPURITY METHYL PARATOLUENE SULFONATE IN LEVOCETIRIZINE DRUG SUBSTANCE BY USING RP-LC METHOD

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ABSTRACT

Highly sensitive method for the determination of genotoxic impurity such as methyl para toluene sulfonate (MPTS) in Levocetirizine drug substance using RP-LC has been presented in the present paper. MPTS were determined by RP-LC method using waters symmetry C18 (250 x 4.6mm), 5µ column as stationary phase. Column temperature maintained 30°C, Injection volume 10µL, flow rare was 1.0 ml/min, sample cooler temperature 5°C and run time was 35mintues. pH 2.50 phosphate buffer was used as mobile phase-A and acetonitrile and water in the ratio of 90:10 %v/v was used as mobile phase-B. The method validation has been carried as per International Conference on Harmonization guidelines (ICH). Limit of quantitation (LOQ) was found 64.446 µg/ mL for MPTS.

Key words: Genotoxic impurity, Levocetirizine, RP-LC method, validation and limit of quantitation.

Abbreviations:

LCZ= Levocetirizine , MPTS=Methyl para toluene sulfonate

1.0 Introduction

Synthesis of drug substances often involves the use of reactive reagents and hence, these reagents may be present in the final drug substances as impurities. Such chemically reactive impurities may have unwanted toxicities, including genotoxicity and carcinogenicity and are to be controlled based on the maximum daily dose [1]. These limits generally fall at low µg/mL levels. HPLC, GC methods (or final drug substance methods) are suitable for their determination. Their applications are oriented towards the potential identification and quantitation of trace level of impurities in drug substances [2].

The assessment and control of Genotoxic impurities (GTIs) in pharmaceutical products has received considerable attention in recent years. The International Conference on Harmonization (ICH), section Q3A, provides guidance on impurities in New Drug Substances [3]. It states that lower reporting thresholds can be appropriate if the impurity is unusually toxic. The European Medices Agency (EMEA) issued guidelines for GTI limits and included the concept of threshold toxicological concern (TTC) to define acceptable risk for the new active substances [4]. Recently, in 2008 US FDA (United States Food and Drug Administration) has also come up with the draft guidelines on genotoxic and carcinogenic impurities in drug substances and drug products [5]. Pharmaceutical Genotoxic impurities may potentially increase the risk of cancer in patients. These guidelines describe ways to characterize, monitor and control the level of GTIs in drug substances and drug products. A maximum daily exposure target of 1.5 µg per day (acceptable Threshold of Toxicological Concern, TTC) is recommended in these guidelines [4–6]. Levocetirizine (LCZ), chemically is (2-[4-[(R)-(4-chlorophenyl) henylmethyl]-1-piperazinyl]ethoxy]-acetic acid dihydrochloride) is a third generation non sedative antihistamine [7] and developed from the second generation antihistamine cetirizine. The chemical structures were represented in (**Figure 1.1).**

Methyl para-Toluenesulfonic acid (MPTS) **(Figure 1.2)**, a strong organic acid, is a common reagent used in pharmaceutical industry as an "organic-soluble" acid catalyst or in purification steps of chemical synthesis of a drug substance. In the manufacturing process of LCZ, methane sulfonic acid (MSA) and para toluene sulfonic acid (PTSA) are used as reagents and alcohol (viz. ethanol) are used as solvent and hence genotoxic methyl paratoluene sulfonate (MPTS) may exist as impurity in LCZ drug substance. Based on maximum daily dose of LCZ (2.5mg/ day), these are to be controlled at a limit of 1.5µg/mL.

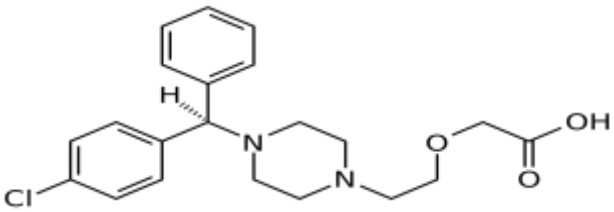

Figure 1.1: Chemical structure of Levocetirizine

1.1 Impurities structures

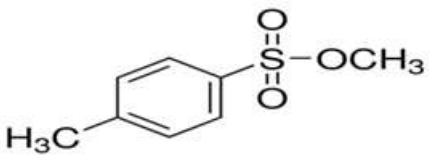

Figure 1.2: Chemical structure of Methyl para toluene sulfonate

Literature survey revealed that LCZ has been reported to be determined by UV Spectrophotometry based on charge transfer reaction [8] LC-MS-MS [9-10] RP-HPLC [11] and by HPTLC [12] were reported for the simultaneous determination of Levocetirizine.

However, no analytical method was reported for the determination of MPTSS in LCZ. Hence the author was aimed towards the development of rapid, specific and robust method for the determination of MPTS in LCZ at trace level concentration.

2.0 Experimental

2.1. Chemicals and reagents

Methyl para toluene sulfonate (MPTS) purchased from Aarti Drugs Ltd., Mumbai, India. Potassium dihydrogen ortho phosphate and acetonitrile were procured from Merck, India. Pure samples of Levocetirizine were obtained from synthetic division of Century pharmaceutical Ltd. (R&D), Vadodara, and Gujarat, India.

2.2 Preparation of solutions

Preparation phosphate buffer pH 2.5

Accurately weighed 6.85 g of potassium dihydrogen phosphate was dissolved in 1000.0 mL of Milli-Q water and adjusted the pH to 2.50 with diluted ortho phosphoric acid. The solution was filtered through 0.45µ filter paper and degassed.

Mobile phase-A preparation

pH 2.50 phosphate buffer was used as mobile phase-A

Mobile phase preparation

Prepared a mixture of 900 mL of acetonitrile and 100 mL of water in the ratio of 90:10 v/v and sonicated to degassed.

Diluent preparation

Prepared a mixture of 600 mL of acetonitrile and 400 mL of water in the ratio of 60:40 v/v and sonicated to degassed.

Preparation of stock solutions

MPTS stock solution was prepared by dissolving 15mg methyl para toluene sulfonate impurity into 50 mL of diluent. Further diluted 0.2 mL of this into 50 mL with diluent. The mixture solution, 300 µg/mL with respect to 4 mg/mL of Levocetirizine. A blend solution was also

prepared by spiking 300 µg/mL of MPTS to 4 mg/mL of Levocetirizine and is used for method development.

2.3 Chromatographic conditions

RP-LC analysis was carried out on Agilent-1200 (Agilent Corporation, USA) wavelength 220 nm. Waters symmetry C18 250×4.6mm, 5µ column was used as stationary phase. The mixture of pH 2.50 phosphate buffer was used as mobile phase-A and acetonitrile and water in the ratio of 90:10 %v/v was used as mobile phase-B. The flow rate of the mobile phase was kept at 1.5mL/min. The injection volume was set as 10µL. Column oven temperature and auto sampler temperature were set as 30°C and 5°C, respectively and run time was 35mintues.

3.0 Results and Discussion

3.1. Method development

A blend solution containing MPTS and Levocetirizine was run in 0.6 mL/min flow rate. Levocetirizine eluted too extended and hence the flow rate of the mobile phase was increased from 0.6 mL/min to 1.0 mL/ min. In this condition Levocetirizine eluted at an optimum retention time, but the retention times of MPTS was drastically increased. Hence, the elution order was observed from the chromatogram **(Figures 1.3 to 1.6)** Levocetirizine solution spiked with MPTS (300µg/mL).

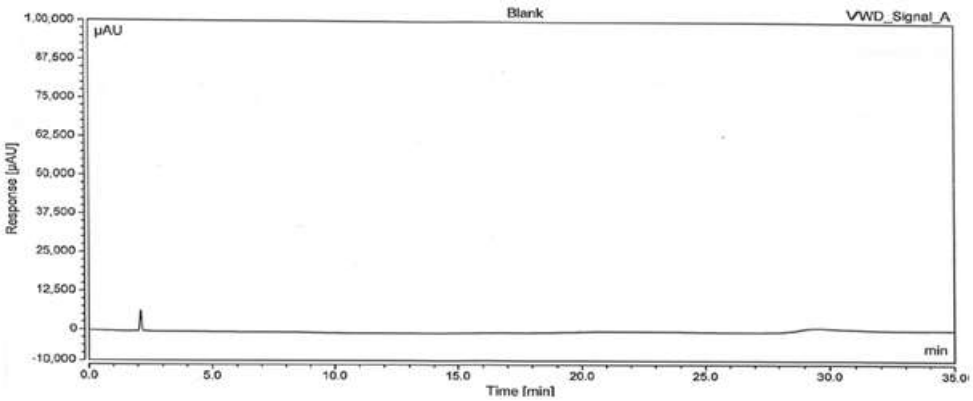

Figure 1.3: Chromatogram of blank

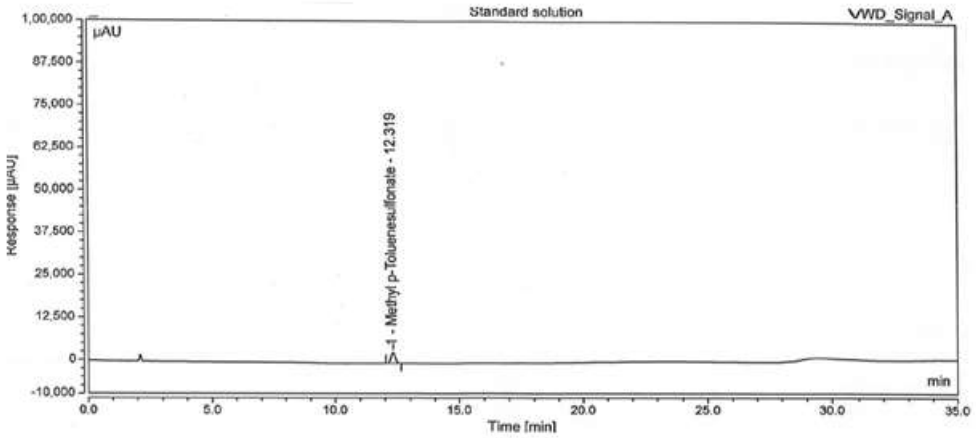

Figure 1.4: Chromatogram of standard solution

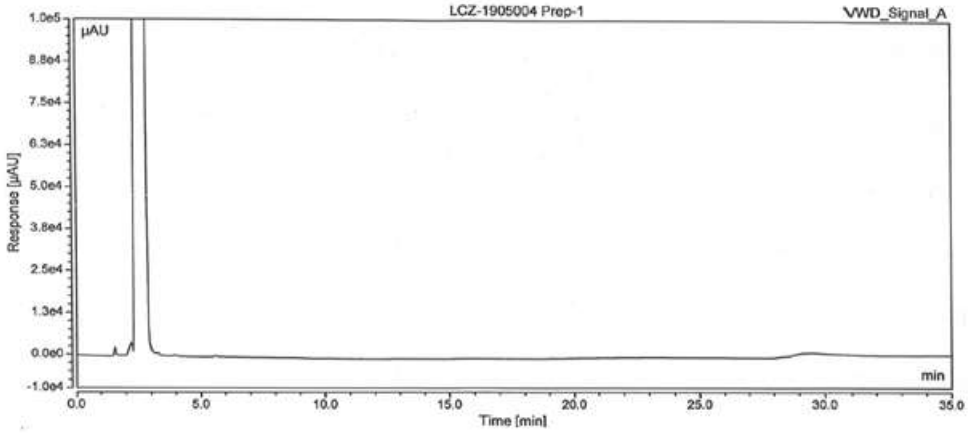

Figure 1.5: Chromatogram of control sample solution

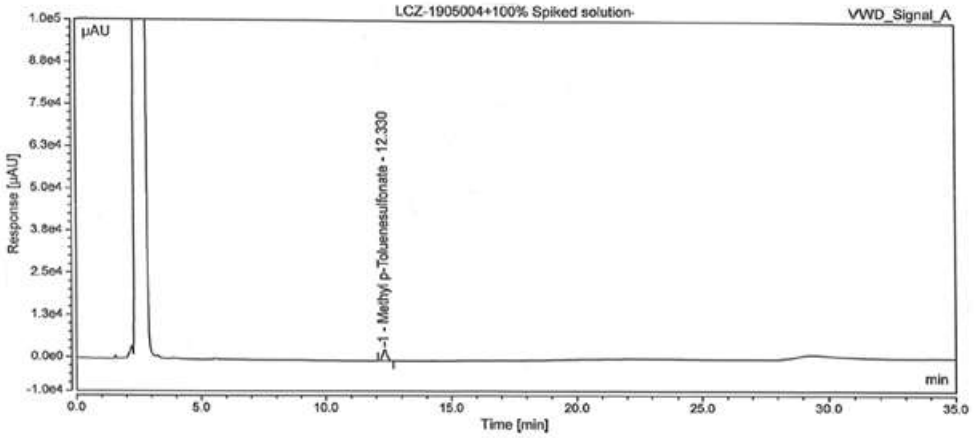

Figure 1.6: Chromatogram of spiked MPTS of sample solution

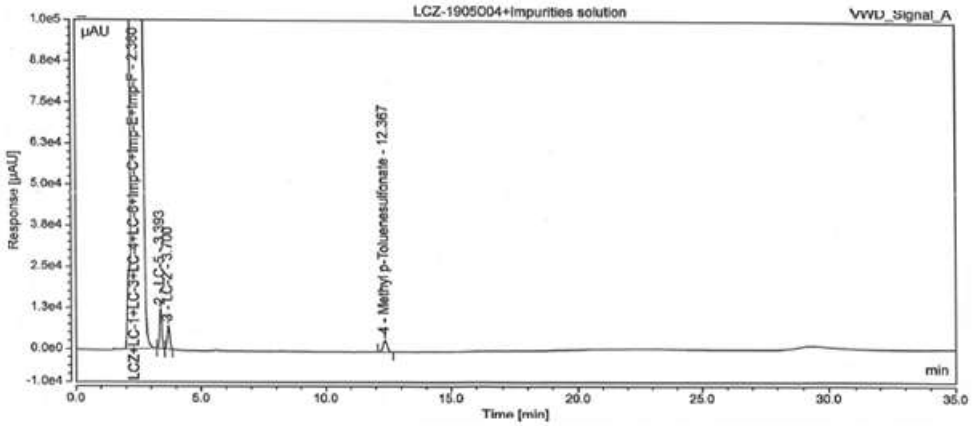

Figure 1.7: Chromatogram of levocetirizine impurities spiked sample solution

3.2. Method validation

3.2.1 Specificity

Selectivity of the method for the estimation of MPTS impurity in drug substance described in the present study was proven by injecting separately solutions of drug substance, other related impurities that are expected to be present in the drug substance and the genotoxic impurity where drug substance and its related impurities eluted below 5 minutes and MPTS impurity eluted after 10 minutes **(Figure 1.7).**

3.2.2 Sensitivity (Limit of detection and Limit of quantification)

Sensitivity was determined by establishing limit of detection (LOD) and limit of quantification (LOQ) for MPTS impurity, which were estimated through signal to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions having a known concentration. LOD of the impurity is defined as the lowest concentration that can be detected. LOD for MPTS impurity was found to be 21.267 µg/ mL. LOQ is the lowest concentration of an impurity that can be quantified with acceptable precision and accuracy. LOQ for MPTS impurity was found to be 64.446 µg/ mL **(Figure 1.8).** Precision study was also carried out at LOQ level by injecting six

individual preparations of MPTS impurity and by calculating the %RSD for the content in µg/mL of impurity.

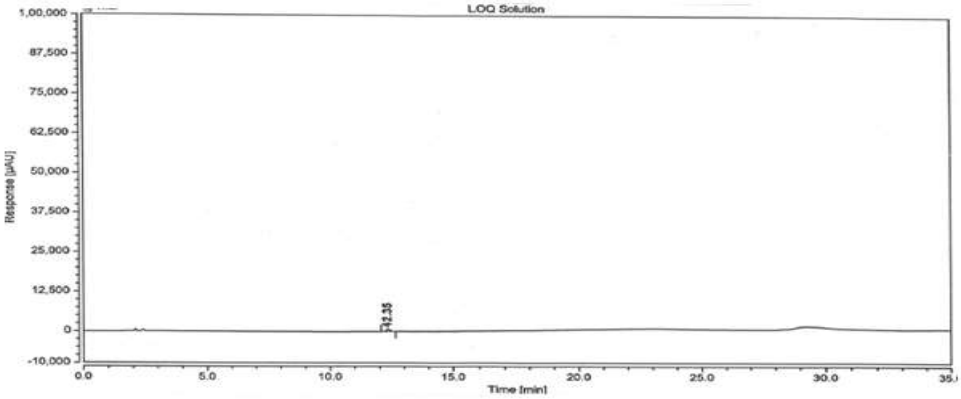

Figure 1.8: Chromatogram of LOQ solution

3.2.3 Precision

Precision of the method was checked by injecting six individual preparations of MPTS impurity at four different levels i.e. LOQ, 50%, 100% and 150% of the specification limit. %RSD was calculated for the content of MPTS impurity in µg/mL. Intermediate precision of the method was also evaluated using three different columns, three different instruments on different days, in the same laboratory.

3.2.4 Linearity

Linearity of the method was demonstrated using a six point calibration plot at concentrations ranging from LOQ to 150% with respect to sample concentration (i.e. 64.4, 161.1, 241.7, 322.2, 402.8 and 483.3 µg/mL). The correlation coefficient of linear regression is greater than 0.9999.

3.2.5 Accuracy

Accuracy of the method was evaluated at four concentration levels in triplicate. Analysis was carried out by spiking the impurity to the drug substance at different concentrations of LOQ, 50%, 100% and 150% of specification limit of MPTS impurity (i.e. 64.4, 161.1, 322.2 and 483.3

µg /mL). The % recoveries were calculated for MPTS impurity which was found to be 99 to 100.3 % respectively.

3.2.6 Batch analysis

Three different lots of Levocetirizine drug substance were analyzed in triplicate in the validated method for estimation of MPTS found that the impurity were below detection Limit (< 21.267 µg/ mL).

3.2.7 Solution stability

Solution stability of standard, control sample and spiked sample was studied up to 24 hrs at room temperature and 2-8°C found no significant difference in the area of standard, control sample and spiked sample was observed from initial to 24 hrs time interval, respectively.

Table 1.0. Validation data of Levocetirizine for the determination of MPTS

Parameter	MPTS
LOD (µg/mL)	21.267
LOQ (µg/mL)	64.446
Precision at LOQ level (RSD, %)	2.10
Method Precision at sixth level (RSD, %)	0.72
Intermediate precision at LOQ (RSD, %)	0.65
Linearity range (µg/mL)	64.4-483.3
Correlation coefficient	0.9999
Slope	107.25
Intercept	122.98
Accuracy at LOQ (recovery)	99.5
Accuracy at 50% (recovery)	100.3
Accuracy at 100% (recovery)	99.5
Accuracy at 150% (recovery)	99.0

4. Conclusion

The proposed RP-LC method that can quantify genotoxic impurity methyl para toluene sulfonate in Levocetirizine at trace level concentration have been developed and validated as per ICH guidelines. The effectiveness of the method was ensuring by the specificity, precision, accuracy and robustness. Hence, the method well suit for their intended purposes and can be successfully applied for the release testing of Levocetirizine into the market.

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Conflict of interests

The authors claim that there is no conflict of interest.

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