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Original Article

METHOD DEVELOPMENT AND VALIDATION BY GC-MS FOR QUANTIFICATION OF 1-CHLOROETHYL CYCLOHEXYL CARBONATE AS A GENOTOXIC IMPURITY IN CANDESARTAN CILEXETIL DRUG SUBSTANCE

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ABSTRACT

Objective: Candesartan Cilexetil is an Angiotension II receptor antagonist used mainly for the treatment of hypertension. The synthesis process of Candesartan uses 1-Chloroethyl Cyclohexyl Carbonate (CECC), which is potential genotoxic impurity as per the EMEA guideline. The method development and later validation activity was proposed and performed for the analysis of 1-Chloroethyl Cyclohexyl Carbonate (CECC) in Candesartan Cilexetil drug substance. Based on daily dose basis evaluation limit required was 0.49 µg/mL (i. e. 49 µg/g).

Methods: The development activity was conducted by Gas chromatography technique with Mass spectrometer as detector. DB-5 make Agilent J&W column with length 50 meter, internal diameter 0.32 mm, film thickness 0.52 µm was used. Phase of DB-5 is 5% phenyl, 95% dimethyl polysiloxane and is an intermediate polarity phase with very good selectivity for polar compounds. Hexane was selected as diluent considering the selective solubility of CECC.

Results: Validation activity was planned and completed based on the International Conference on Harmonization (ICH) guidelines. The LOD and LOQ values were found to be 0.70 and 2.11 µg/g (i. e. 0.007 and 0.021 µg/mL) for CECC. Method very sensitive as method was related to genotoxic impurity. Also accuracy results were well in the range of 99 to 105 %. Linearity curve showed correlation coefficient of 0.99997.

Conclusion: From validation data it was confirmed that the method is specific, precise, accurate, linear and sensitive for the required purpose. During the same activity three commercial batches were analyzed. Validation data as well as commercial batch data was enough to prove the method suitability.

Keywords: Candesartan Cilexetil, 1-Chloroethyl Cyclohexyl Carbonate, Gas chromatography, Mass spectrometer, Limit of Quantification (LOQ), Limit of Detection (LOD).

INTRODUCTION

1-Chloroethyl Cyclohexyl Carbonate is a potential genotoxic impurity in Candesartan Cilexetil drug substance as it was the part of synthesis process. As per the International conference on harmonisation Guidelines from European medical Agency the genotoxins were to be limited to 1.5µg/day [1, 2]. Based on the data of daily dose the evaluation limit was set as 49 µg/g of drug substance i. e. as such 0.49µg/mL for analysis). Since 1-Chloroethyl Cyclohexyl Carbonate is having -chloro as a functional group with aliphatic chain, as per the guideline it is a genotoxic alerting compound. Sensitive method for the analysis of CECC as genotoxic impurity was not available. While developing method at such a low-level, interferences due to drug substance as well as other process impurities and degradation products were the major problems in achieving specificity. Hence based on published general strategies for genotoxic impurities and on the threshold of toxicological concern (TTC), CECC was evaluated in Candesartan Cilexetil drug substance [3, 4 and 5].

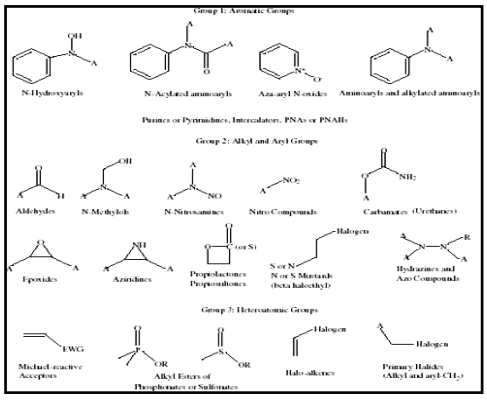
General Genotoxic alerting structures

Candesartan is chemically known as 2-Ethoxy-3-[21-(1H-tetrazol-5-yl) biphenyl-4-ylmethyl] -3Hbenzoimidazole- 4-carboxylic acid 1-Cyclohexyloxy Carbonyloxy ethyl ester [6]. Method was developed with Gas chromatographic technique and Mass spectrometer as detector for CECC in Candesartan Cilexetil drug substance. Parameters to be performed for the validation of developed method are decided as per the guideline of International Conference on Harmonization (ICH) [7, 8].

MATERIALS AND METHODS

Materials

Analytical grade reagents were used in method development and validation activity. Three batches of Candesartan Cilexetil drug substances for method development and analysis were used. 1-Chloroethyl Cyclohexyl Carbonate (CECC) was procured from Gensynth Fine Chemicals. Hexane was used as diluent for the preparation of Standard and sample solutions and was purchased from Merck Chemicals.



GC-MS operating conditions

Candesartan Cilexetil drug substance samples were analyzed for CECC content with before validated method. The analysis was performed with Shimadzu GCMS QP-2010 with Quadrupole mass analyzer with auto injector Shimadzu AOC-20i and software control used was GCMS solution version 2.61. Analysis was performed on column DB-5 make Agilent J&W, with length 50 meter, internal diameter 0.32 mm, film thickness 0.52 µm. Injection volume of 1 µL was used and split ratio was kept at 1:15. In GC column oven

programme initial temperature 180°C and initial hold time was kept 3 min, then the temperature was raised to 300°C at the rate 10°C/minute and the final temperature hold was kept 7 minutes. Injector temperature was maintained at 280°C. Ion source temperature was kept at 200°C, while Interface Temperature was 280°C. Helium was used as the carrier gas with column flow rate 29.7 cm/sec. Ionization energy used for the optimum ionization was 70 eV.For identification of CECC peak full scan was conducted in the range 10-500 amu and a spectrum was used for the identification. GCMS solution software version 2.61 was used for the mass spectral analysis. Compound was identified using the reference spectra in the library of National institute of Standard Technology.

Solvent cut time was kept at 0.0 min to 4.0 min and acquisition time was kept at 5.1 mine to 6.8 min. Analysis was conducted in selective ion monitoring mode (SIM mode).

Preparation of solutions for analysis

1-Chloroethyl Cyclohexyl Carbonate solution was prepared diluting 62 mg of standard material to 50 mL using Hexane as diluent. Further 1.0 ml of the resulting solution was diluted to 50 mL with diluent (23.6µg/mL).

Again further 1.0 ml of the above solution was diluted to 50 mL (0.49µg/mL).

Sample solution was prepared by diluting the 50 mg of Candesartan Cilexetil sample to 5.0 mL with Hexane which was used as diluent.

LOD-LOQ prediction solutions were prepared in the concentration range from 0.023µg/mL to 0.35µg/mL. Also the Linearity solutions were prepared between the concentrations ranges from LOQ and1.25µg/mL. Hexane was used as diluent as well as injected as blank during the analysis and validation activity.

RESULTS AND DISCUSSION

Method development

Method development was planned for the analysis of 1-Chloroethyl Cyclohexyl Carbonate using Gas Chromatographic technique with Mass spectrometer as detector, as evaluation limit was as low as 49µg/g. Different columns with different stationary phases and dimensions were used like DB-1, Rtx-624 and DB-624, Rxi-1301 for the analysis of CECC. Finally the column DB-5 with dimensions 50m length, 0.32 mm internal diameter and 0.52 µm film thickness was selected. It is mid polar column with 5% phenyl and 95% Dimethyl polysiloxane as a Stationary phase. During the method development using this column proper peak shape as well as peak response was obtained.

CECC being high boiling point compound the initial column oven temperature was decided to be 180°C with hold time of 3.0 min then further raised to 300°C at the rate 10°C/min and hold for 7.0 min. Mass spectra of CECC shows base peak at m/z 82 hence it was selected for the quantification using SIM mode. During development no interference from the sample matrix was observed due to use of SIM mode as well as sensitivity was also enhanced. The method was developed and validated with necessary parameters.

Method validation

Method validation activity was conducted with incorporation of necessary parameters as per the Analytical method validation guidelines of International conference on Harmonisation. Specificity was completed by injecting other solvents used during the manufacture of Candesartan Cilexetil drug substance. As no interference was observed at the retention of the analyte CECC, method was specific.

System suitability

Further the standard solution of CECC was injected six times as a part of system suitability and further different concentrations ranging from 0.022 to 1.25µg/mL were injected into the system for performing different studies.

Limit of detection and limit of quantification

LOD and LOQ values were established using calibration curve method i. e. using slope and intercept of the calibration curve LOD and LOQ values were established. The established values of LOD and LOQ are then proved for precision by injecting six injections of each concentration. LOD and LOQ values proved for CECC in this method are 0.007 and 0.021µg/mL.

Linearity

For conducting the linearity study concentration range from 0.022 to 1.25µg/mL was considered which was from LOQ to 250% of the evaluation limit. Calibration curve was plotted between the peak areas against the concentration of CECC (y= 7934. x + 108.2). Correlation coefficient observed for linearity study was 0.99997.

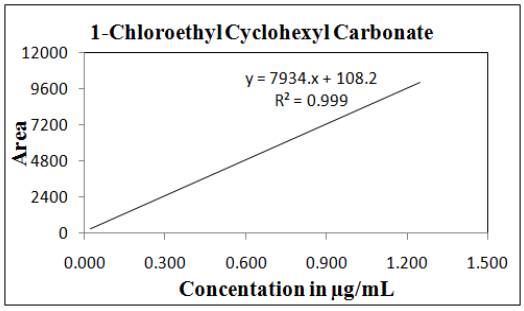


Fig. 1: Linearity graph of 1-Chloroethyl Cyclohexyl Carbonate

Precision and accuracy

Percentage relative standard deviation observed for six replicate injections of standard during system precision study was observed to be 2.2, which was well within the acceptance criteria. Low RSD value of peak areas of analyte confirms the precision of the developed method. Accuracy was performed by spiking the samples of Candesartan Cilexetil drug substance at 0.247, 0.494 and 0.741 µg/mL concentrations which was 50%, 100% and 150% of the evaluation limit. The average percentage of recovery was observed well within the acceptance criteria as provided in table 1.

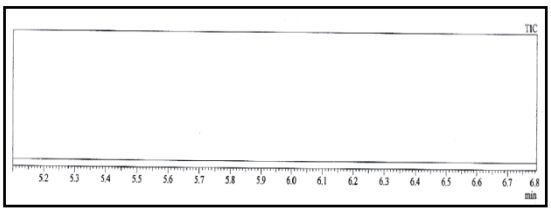


Fig. 2: GC-MS TIC of Blank

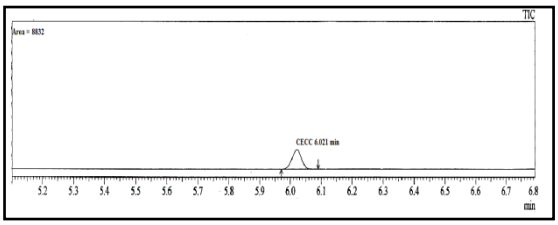


Fig. 3: GC-MS TIC of 0.49 µg/mL concentration of 1-Chloroethyl Cyclohexyl Carbonate.

Further to validation activity conducted three batches of Candesartan Cilexetil drug substance were analyzed and CECC was observed to be not detected in all the three batches.

As per the validation parameters performed i. e. Specificity, Limit of detection, Limit of Quantification, Linearity, Precision and Accuracy, all the results observed are well within the set acceptance criteria. Hence based on the validation activity and further analysis of commercial batches, it was confirmed that the method was suitable for use in routine analysis.

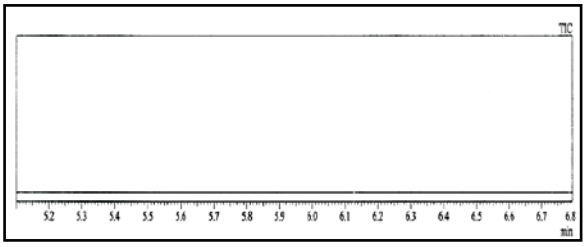


Fig. 4: GC-MS TIC of Candesartan Cilexetil Plain Sample

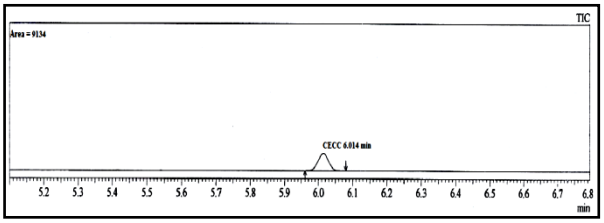


Fig. 5: GC-MS TIC of Candesartan Cilexetil Spiked Sample

Mass spectral analysis

As per the analysis conducted on GC-MS and the retention time of CECC was in the range 5.5 to 6.5 minutes as shown in the fig. 2. The Mass spectrum of 1-Chloroethyl Cyclohexyl Carbonate was as shown in fig. 5. As shown in the spectrum of CECC, the parent peak was observed at 207 which confirm the molecular formula $C_9H_{15}O_3Cl$. Major fragments are observed at 119, 133, 96, 82, 67, 44 and 32. The spectrum observed matches exactly to the reference spectrum.

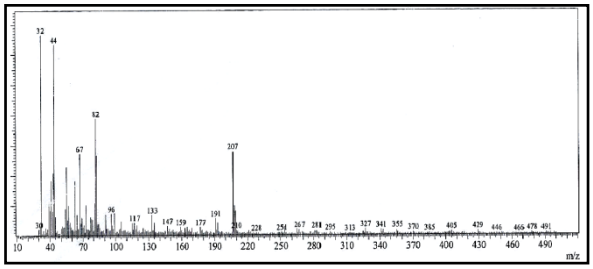
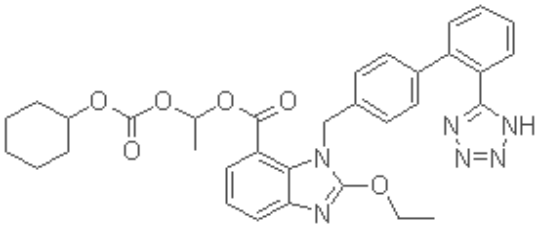
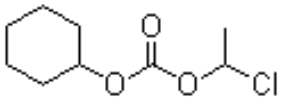


Fig. 6 Mass spectrum of 1-Chloroethyl Cyclohexyl Carbonate



Structure of Candesartan Cilexetil



Structure of 1-Chloroethyl Cyclohexyl Carbonate

Table 1: Recovery data of 1-Chloroethyl cylcohexyl carbonate

Candesartan Cilexetil drug substance spiked with CECC	Recovery of 1-Chloroethyl Cyclohexyl Carbonate, % (mean ±difference)		
	0.247 µg/mL level	0.494 µg/mL level	0.741 µg/mL level
sample preparation-1	103.6±0.1	104.0±1.5	100.1±0.1
sample preparation-2	102.7±0.8	102.0±0.5	100.8±0.6
sample preparation-3	104.1±0.6	102.5±1.0	99.8±0.4

CONCLUSION

Method was developed and validated for the content of CECC in Candesartan Cilexetil drug substance using gas chromatographic technique and mass spectrometer as detector. Method is very sensitive with LOD and LOQ values at 0.007µg/mL and 0.021µg/mL respectively.

The Relative standard deviation values of precision data show that method is precise enough to reproduce the results. Linearity correlation coefficient was observed to be 0.99997. Hence, after studying the data obtained from the validation activity it was established that the method was best suited for the use in regular analysis purposes.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

Declared none

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