Determination of genotoxic impurity chloromethyl chloroformate in tenofovir disoproxil succinate using RP-LC

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Abstract

Highly sensitive method for the determination of genotoxic impurity such as chloromethyl chloroformate) in tenofovir disoproxil succinate using RP-LC has been presented in the present study. Quantification of CMCF (Chloromethyl chloroformate) content in Tenofovir disoproxil succinate samples was made by HPLC with UV Detector. CMCF was UV inactive compound. Derivatization procedure was established to detect the CMCF in HPLC. For this 1-(3-Methyl-1-phenyl-1H-pyrazol-5-yl), piperazine was used as a derivatizing agent which reacts with CMCF in the presence of Triethyl amine to form a compound which was UV active. CMCF was determined by RP-LC method using X-Terra MS C18 (250X4.6mm, 5µm) column as stationary phase.

Column temperature maintained 35°C, Injection volume 20µL, Flow rare was 1.0 ml/min, sample cooler temperature ambient and run time was 60mintues. pH 3.00 phosphate buffer is used. The mixture of buffer and acetonitrile in 64:36 (v/v) was used as mobile phase. The method validation has been carried as per International Conference on Harmonization guidelines. Limit of quantitation (LOQ) was found 2.48ppm for CMCF.

Keywords: Genotoxic impurity, Tenofovir disoproxil succinate, RP-LC method, validation and limit of quantitation.

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 chemical name of tenofovir disoproxil succinate is 9-[(R)-2-[[bis(isopropoxycarbonyl)oxy]methoxy] phosphinyl] methoxy] propyl] adeninesuccinate.[3,4] Corresponding to the molecular formula $C_{23}H_{36}N_5O_{14}P$, it has a relative molecular mass of 637.5 g/mol. Tenofovir disoproxil succinate is a white to off-white, not hygroscopic crystalline powder. It is slightly soluble in water and sparingly soluble to soluble in aqueous media across the physiological pH range. Its pKa has been found to be 4.87 and its partition coefficient was found to be 0.94. Tenofovir disoproxil succinate is known to exhibit polymorphism. Tenofovir is an adenine analog reverse transcriptase inhibitor with antiviral activity against HIV-1 and hepatitis B. It is used to treat HIV infections and chronic hepatitis B in combination with other antiviral agents, due to the emergence of antiviral drug resistance when it is used alone.

In the manufacturing process of tenofovir disoproxil succinate, chloromethyl chloroformate (CMCF) is used as reagent and hence genotoxic chloromethyl chloroformate (CMCF) may exist as impurity in tenofovir disoproxil succinate drug substance.

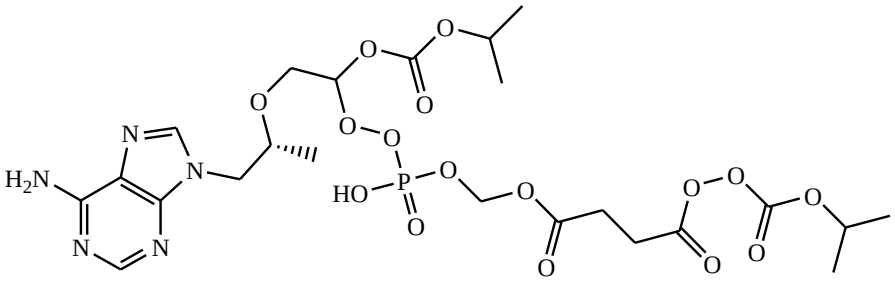


Fig. 1: Chemical structure of Tenofovir disoproxil succinate

Impurity structure:

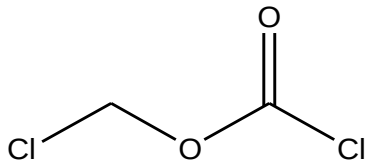
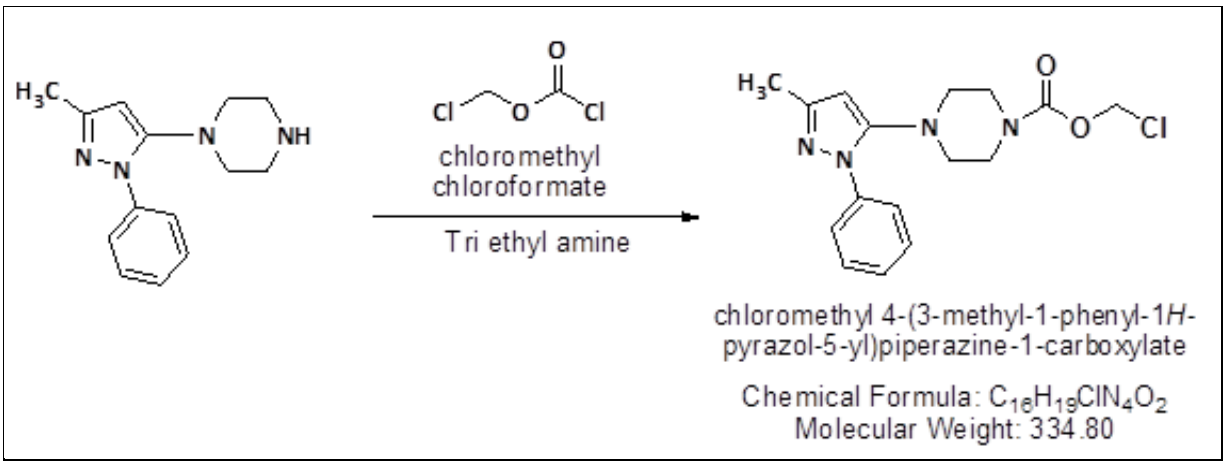


Fig. 2: Chemical structure of Chloromethyl Chloroformate (CMCF)

Chloromethyl chloroformate was UV inactive compound. Derivatization procedure was established to detect the chloromethyl chloroformate in HPLC. For this 1-(3-Methyl-1-phenyl-1H-pyrazol-5-yl) piperazine was used as a derivatizing agent which reacts with CMCF in the presence of triethyl amine to form a compound chloromethyl 4-(3-methyl-1-phenyl-1H-Pyrazol-5-yl)piperazine-1-carboxylate which was UV active.



Material and Methods

Chemicals and reagents: Chloromethyl chloroformate was (CMCF) purchased from Sigma-Aldrich., Mumbai, India. Potassium dihydrogen ortho phosphate and acetonitrile were procured from Merck, India.

Buffer: Dissolve 1.36g of KH_2PO_4 in 1000 mL of Milli-Q-water, and adjust to pH 3.0±0.05 with ortho-phosphoric acid. Filter through 0.45µ membrane filter paper.

Preparation of diluent: Dissolve 400.966mg of 1-(3-Methyl-1-phenyl-1H-pyrazol-5-yl) piperazine in 2000 mL of Acetonitrile. To this add 2.0 mLof triethyl amine and mixed well.

Preparation of CMCF stock solution: Transfer 9µL of CMCF into a 50mL volumetric flask containing about 30mL of Acetonitrile. Mix well and make up to the mark with Acetonitrile.

Standard solution: Transfer 40µLof CMCF stock solution into a 50mL volumetric flask containing about 30mL of diluent. Mix well and make up to the mark with diluent. This solution is equivalent to 5.0ppm of CMCF with respect to 40.0mg/mL of sample solution.

Preparation of sample spiked solution: Weigh 400mg of the Tenofovir disoproxil succinate into a 10mL volumetric flask. Dissolve in 5mL of diluent and add 8µL of CMCF stock solution. Mix well and then make up to the mark with diluent.

Chromatographic conditions: RP-LC analysis was carried out on Agilent-1200 (Agilent Corporation, USA) wavelength 210 nm. X-Terra MS C18 (250X4.6mm, 5µm) column was used as stationary phase. The mixture of pH 3.00 phosphate buffer and acetonitrile in the ratio of 64:36 (v/v) was used as mobile phase. The flow rate of the mobile phase was kept at 1.0mL/min. The injection volume was set as 20µL. Column oven temperature and auto sampler temperature were set as 30°C and 25°C respectively.

Results and Discussion

Method development: A blend solution containing CMCF and tenofovir disoproxil succinate was run in 1.5 mL/min flow rate. Tenofovir disoproxil succinate eluted too extended and hence the flow rate of the mobile phase was decreased from 1.5 mL/min to 1.0 mL/ min. In this condition tenofovir disoproxil succinate was eluted at an optimum retention time, but the retention time of CMCF was drastically increased. Hence, the elution order was observed from the chromatogram (Fig. 5) Tenofovir disoproxil succinate solution spiked with CMCF (5µg/mL).

Method validation: The developed method was validated as per ICH guidelines[5] in terms of specificity, limit of detection (LOD), limit of quantitation (LOQ), precision, linearity, accuracy and system suitability and the data are presented in table 1.

The specificity of the developed LC method was indicated by CMCF solution (5µg/mL each) with respect to 40mg/mL of TDS injected separately and S/N ratios were recorded. These solutions were further diluted to achieve the signal-to-noise (S/N) ratios at about 3 and 10 for determining LOD and LOQ, respectively for both the methods.

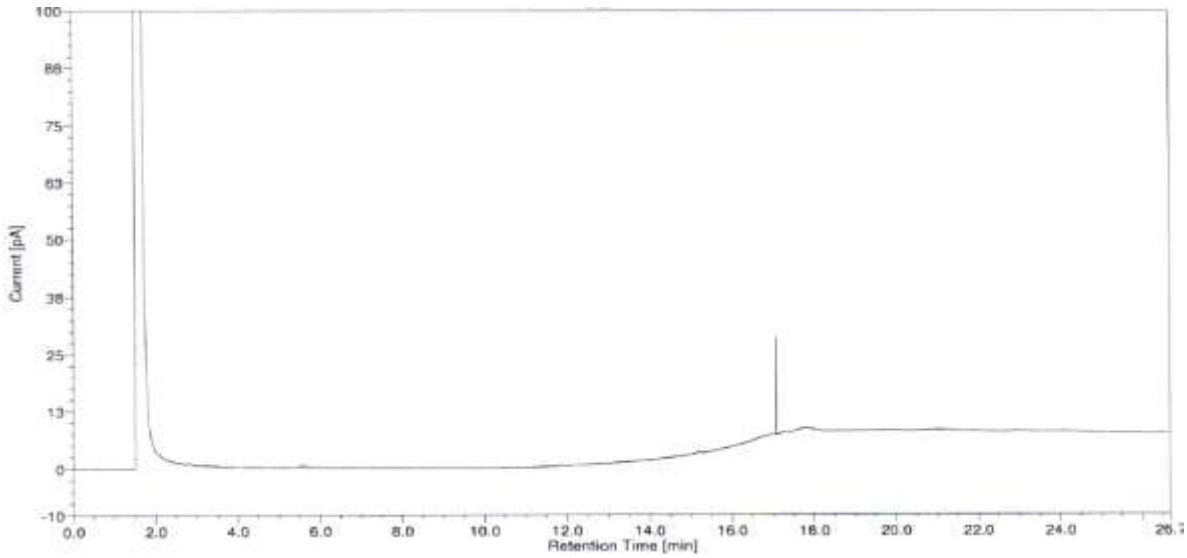


Figure 3: typical chromatogram of Blank

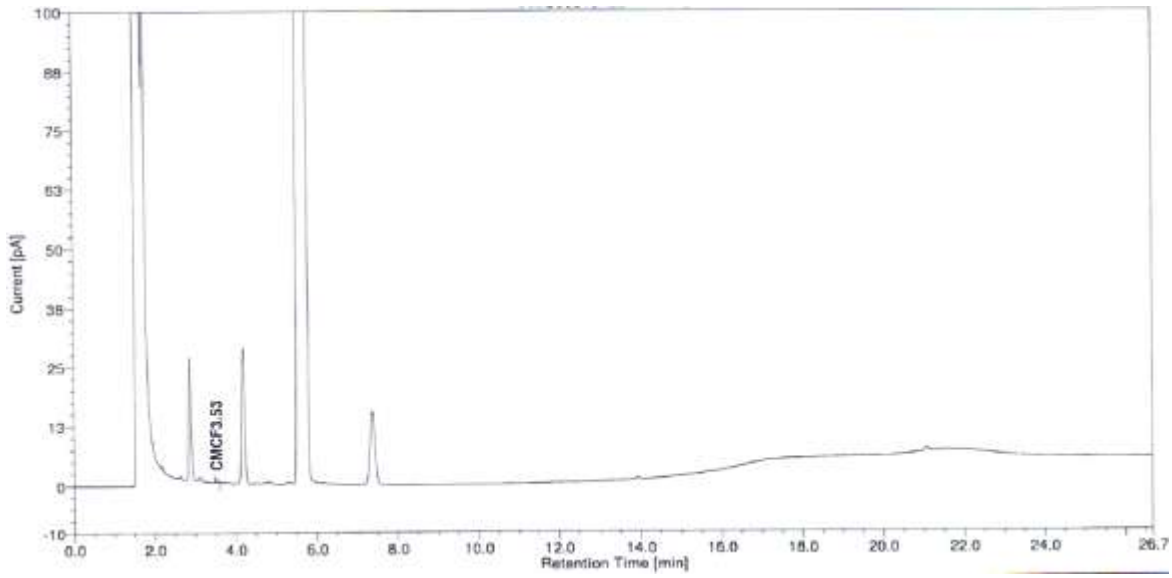


Figure 4: typical chromatogram of Tenofovir disoproxil succinate

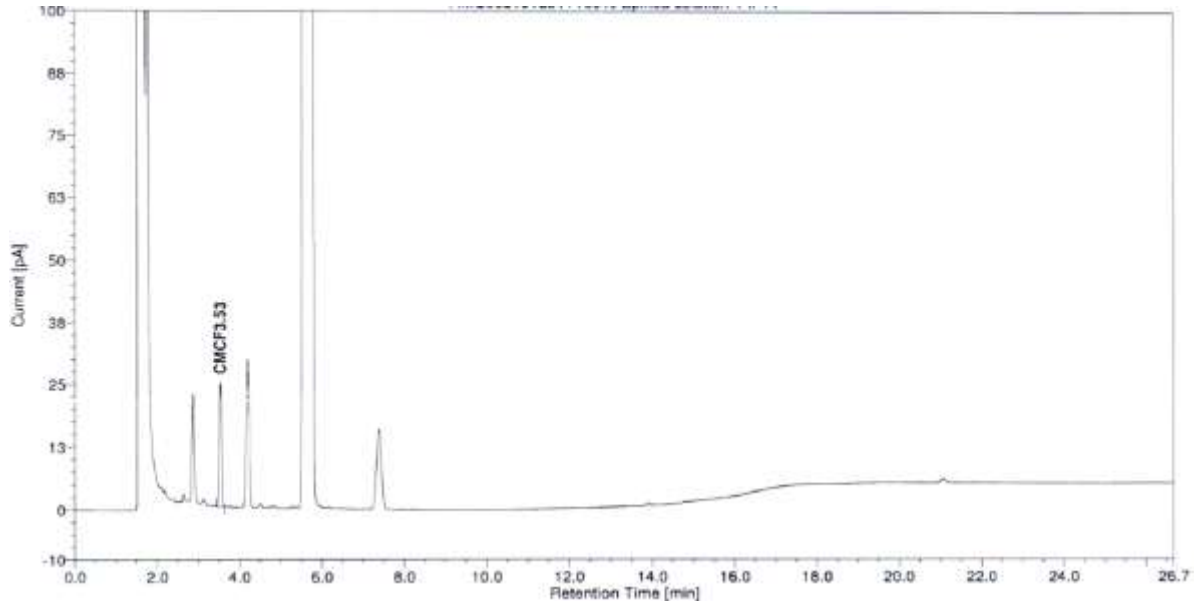


Figure 5: Spiked CMCF chromatogram of Tenofovir disoproxil succinate

The precision of the methods was checked by injecting LOQ solutions for six times. The value of RSD for area of CMCF was calculated.

Table 1
Validation data of Tenofovir disoproxil succinate for the determination of CMCF

Parameter	CMCF
LOD (µg/mL)	0.85
LOQ (µg/mL)	2.48
Precision at LOQ level (RSD, %)	2.45
Precision at sixth level (RSD, %)	2.34
Intermediate precision at LOQ (RSD, %)	1.36
Linearity range (µg/mL)	LOQ-200
Correlation coefficient	0.9990
Slope	6584.60
Intercept	-536.36
Accuracy at LOQ (recovery, %)	97.8
Preparation-1	97.0
Preparation-2	99.0
Preparation-3	97.5

The intermediate precision of the method was also verified on six different days in the same laboratory using the LOQ level solutions. The low RSD values ensured the precision of the developed method. Linearity test solution for CMCF was prepared individually at six concentration levels in the range of LOQ to 200% of the specification level 5µg/mL. LOQ and sixth levels were injected six times and other four levels were injected thrice. The average peak areas versus concentrations were subjected to least-squares linear regression analysis. The derived correlation coefficients were above 0.999 indicating the best fitness of the linearity curves of the developed method.

Standard addition experiments were conducted in triplicate preparations to determine accuracy of the methods at LOQ level and recoveries of all the genotoxins were determined. The recoveries were found to be in the accepted range. The system suitability of the method was ensure by getting the %RSD less than 10.0 for six injections of the CMCF in RP-HPLC method at specification level. Tenofovir disoproxil succinate at trace level concentration have been developed and validated as per ICH guidelines.

Conclusion

The proposed RP-LC method that can quantify genotoxic chloromethyl chloroformate in tenofovir disoproxil succinate at trace level concentration has been developed and validated as per ICH guidelines. The effectiveness of the method was ensured by the specificity, precision and accuracy. Hence, the method is well suited for their intended purposes and can be successfully applied for the release testing of tenofovir disoproxil succinate into the market.

Acknowledgement

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