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Determination of glycidol as a genotoxic impurity in linezolid drug substance by gas chromatography

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ABSTRACT

A gas chromatographic method has been developed for the determination of Glycidol in Linezolid drug substance. The method was optimized based on the basis of peak shape and response of Glycidol. The method was validated as per ICH guideline in terms of LOD, LOQ, Method precision, accuracy and specificity. The LOD and LOQ values were found to be 3 ppm (3 µg/ml) and 10 ppm (10 µg/ml) respectively.

KEYWORDS

Development,
Validation;
Glycidol;
Linezolid;
Gas chromatography.

INTRODUCTION

Linezolid is a synthetic antibiotic used for the treatment of serious infections caused by Gram-positive bacteria that are resistant to several other antibiotics. A member of the oxazolidinone class of drugs, linezolid is active against most Gram-positive bacteria that cause disease, including streptococci, vancomycin-resistant enterococci (VRE), and methicillin-resistant Staphylococcus aureus (MRSA)

Glycidol is an organic compound that contains both epoxide and alcohol functional groups. Being bifunctional, it has a variety of industrial uses. Glycidol is a used as a raw material for Glycidyl butyrate, which was used as a key raw material in the manufacturing of Linezlid drug substance. Glycidol is listed as an IARC group 2A carcinogen, meaning that it is "probably carcinogenic to humans". In regards to occupational exposures, the Occupational Safety and Health Administration has set a permissible exposure limit at 50 ppm over an eight hour work shift, while the National Institute for Occupational Safety and Health recommends a limit at 25 ppm over an eight hour work shift.

Various methods in the literatures involve determination of Linezolid in human plasma(1-2), in dosage form by HPLC(3-8) and by UV(9-13). However no method is available for content of Glycidol in Linezolid by Gas chromatography. In the present work we have developed a new, simple precise, accurate method for determination of Glycidol in Linezolid by gas chromatography in bulk drug.

Structure of Linezolid and Glycidol are shown in Figure1 and Figure 2.

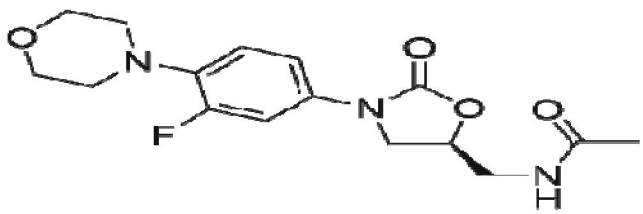


Figure 1

Full Paper

Figure 2

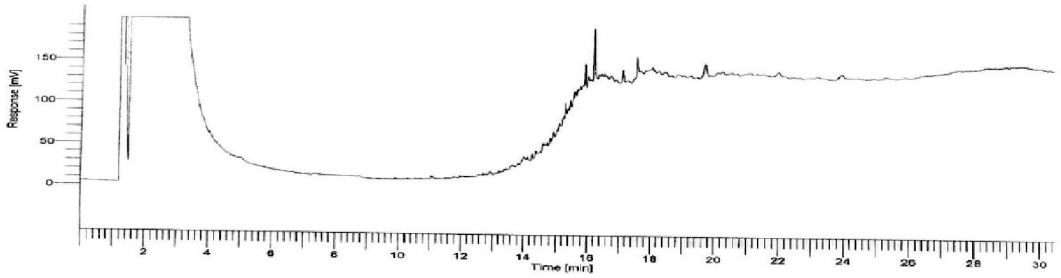
EXPERIMENTAL

Chemicals

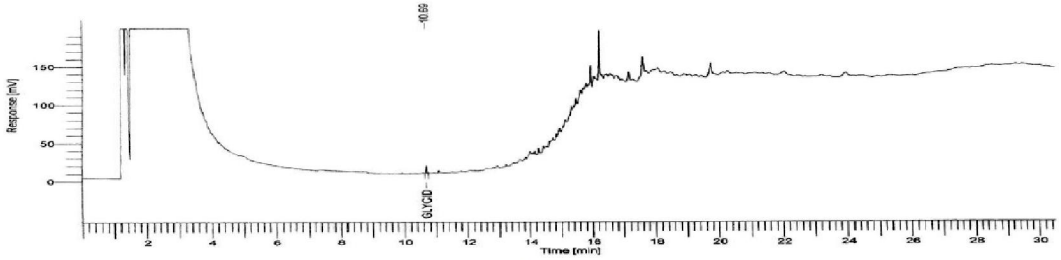
Glycidol was purchased from Aldrich chemicals; Emplura grade cyclohexane was purchased from Merck. Samples of Linezolid are received from local market.

Chromatographic conditions

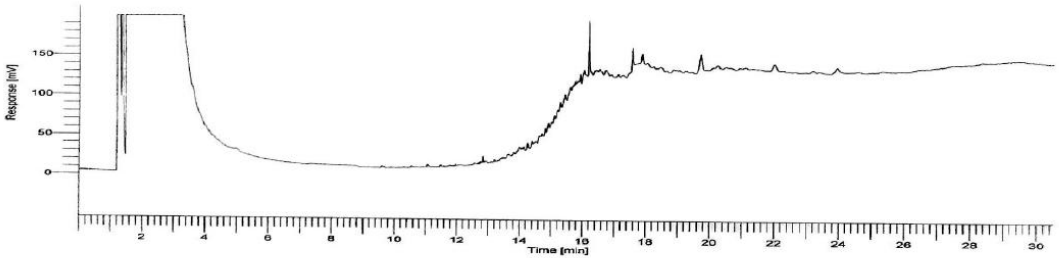
Analysis was carried on a GC system equipped with liquid auto sampler (Perkin Elmer 580 gas chromatograph with auto sampler) with Flame ionisation detector and Helium as a carrier gas. Analysis was done on DB-FFAP (phterethalic acid modified polyethylene glycol phase) column of 30 m length, 0.53 mm inner diameter and 1µm film thickness. Injector temperature and detector temperature were kept as 220°C and 280æ%C respectively. The detector attenuation is kept at -6(1) for maximum response. After optimisation the split was decided as 5mL/min for 5µL injection. Flow of carrier gas (Helium) is kept



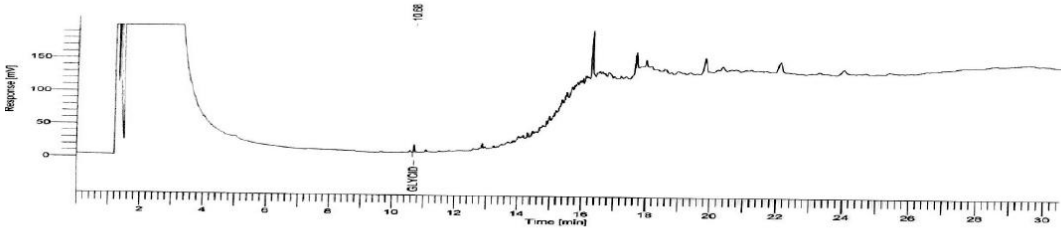
(a)



(b)



(c)



(d)

Figure 4 : Typical chromatograms of (a) Blank (b) Standard (c) Sample (d) spiked sample

at 5.0 mL/min at constant flow. The GC oven temperature is programmed as as 70°C for 7 minutes then raised to 240°C at a rate of 20°C/min and hold for 15 minutes.

Standard stock preparation

Glycidol solution was prepared by diluting 50 mg of Glycidol to 50 ml with Dichloromethane. Further 1 ml of this solution was diluted up to 100 ml with Dichloromethane.

Standard preparation

Further 5 mL of this solution was diluted up to 100 mL with Dichloromethane.

Sample preparation

Weighed about 50 mg of Linezolid sample into a 5mL volumetric flask, added 2mL diluent, sonicate to dissolve and make upto mark with diluent. Filter through 0.45µ Nylon 66 filter and inject.

RESULTS AND DISCUSSION

Method development and optimization

Glycidol is a slightly viscous liquid at ambient temperature with a boiling point of 167°C Solvents used for development were Methanol, Acetone and Dichloromethane. Dichloromethane is finalised as the diluent as Glycidol shows good response compared to Methanol and Acetone. Methanol shows tailing for the peak while Acetone shows interference. The experiment was initially carried out on DB-1 and DB-5 column but was replaced by DB-FFAP column for sharper peak and retention time. The effect of injection volume and split was observed and optimised up to 5 µL with a split of 5mL/min for sample concentration of 10mg/mL.

Method validation

The method validation work was conducted according to the ICH guidelines(14-15). The Validated method parameters include specificity, accuracy, Sensitivity, Precision, linearity, robustness, ruggedness and solution stability. LOD, LOQ values were obtained by preparing a series of known concentration solutions of increasing concentration and plotted a graph of concentration against area of analyte. LOD and LOQ values were found to be 6 ppm and 20 ppm respectively. Linearity of the method was determined by preparing and analyzing a series of 7 standard solutions to cover the concentration range of LOQ to 75 ppm for Glycidol. The linearity correlation coefficient was found to be 0.9991

The method is precise which is indicated by the low % relative standard deviation of six replicate standards, which was 3.32. The accuracy of method was determined by spiking the samples at 50 %, 100 % and 150 % level.

CONCLUSIONS

A simple and sensitive GC method has been developed and validated for the trace analysis of Glycidol in pharmaceuticals. The validation has been conducted according to ICH guidelines. This method is sensitive enough to detect 3 ppm and quantify 10 ppm level of Glycidol in pharmaceutical drug substances.

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