

# GC-FID-HS method development and validation for quantitative estimation of ethanol as residual in tablets drug product from the class of benzodiazepines

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## Introduction

The purpose of this study is to explain why and how to give an understandable concept for the implementation of a trustworthy GC-FID-HS method which will be used for the determination of residual ethanol in solid dosage form (tablets). The tablets drug product belongs to a class of medications benzodiazepines which are used to treat panic attacks with or without agoraphobia, anxiety states of different severity, including those associated with depression, agitation, restlessness and tension accompanied with or without psychosomatic symptoms as well as anxiety which accompanies depressive disorder.

As a central nervous system depressant, ethanol is one of the most commonly consumed psychoactive drugs. Ethanol serves many purposes in medicines, as a solvent, preservative, or cutaneous penetration enhancer. Although, ethanol can impair cognitive and psychomotor functions so exposure to ethanol when used as an excipient in medicines is usually limited. Exposure is affected by the dose volume and weight of the patient. This solvent was evaluated for its possible risk to human health. Therefore, the analysis of ethanol is a necessary tool for the standard management of prescribed drugs (Umamheswari et al., 2021). The concentration of residual ethanol should not exceed the limit prescribed in the ICH guidelines for Class 3 residual solvents (ICH Q3C (R6), 2018; ICH Q3C (R8), 2020). Ethanol anhydrous was used for the preparation of binder solution during the manufacturing process of tablets but it cannot be completely removed by common manufacturing techniques so it was necessary to validate sensitive and accurate method for estimation of ethanol. Some methods according to several literary data, have been established for

the analysis of ethanol, using a mix of several diluents as dissolving solutions (Alahmad et al., 2013) or solid phase microextraction headspace method (Hanwar et al., 2022). However, it is taking too much time and money. This GC-FID-HS method is considered to be a less expensive and fast method for the estimation of ethanol in tablets and it has been widely employed in the analysis. The method was validated according to ICH guidelines Q2 (R2), following various parameters such as specificity, linearity, precision, accuracy, robustness, the limit of quantification (LOQ) and the limit of detection (LOD).

The objective of the development and validation of an analytical procedure is to show that it is suitable for the intended purpose (ICH, 2022).

## Materials and methods

Analytical standard ethanol as well as dimethyl sulfoxide (utilized as diluent in order to increase the method sensitivity and efficiency), were pharmaceutical grade (purity GC 99.9%).

Development and validation of the method was in accordance with the recommendations of the relevant guidelines. A number of preliminary experiments were performed on a Shimadzu GC system equipped with a flame ionization detector (FID) and AOC-5000 Headspace autosampler, using an appropriate capillary column DB-624 (30 m x 0.32 mm x 1.8 µm) stationary phase for chromatographic separation. A selected temperature gradient was applied starting with an initial temperature of 45°C up to 230°C at a rate of 15°C/min, using helium as a carrier gas with a flow rate of 1.5 ml/min. Constant temperatures of the injector (170°C) and detector (250°C)

were applied. The standard solution was prepared in appropriate concentration, then 5.0 mL of solution was taken into a HS vial for analysis. Depending on the dosage form of the tablets (0.25 mg/g, 0.5 mg/g or 1 mg/g), 8, 4 or 2 tablets were taken at random from selected sample tablets and transferred to a HS vial. 5.0 mL of DMSO was added to a vial.

## Results and discussion

The applicability of the proposed method was evaluated by analyzing the samples of tablets in three dosage forms 0.25 mg/g, 0.5 mg/g and 1 mg/g. Implementation of the precise, validated method enables estimation of ethanol as Class 3 residual solvent in tablets within the permitted limits as per USP <467> and Q3C Guidance: 50.0 mg/day daily dose or concentration of 5000 ppm. The system suitability tests were carried out by injecting a single injection of blank solution DMSO, then the standard solution in 6 replicates and the obtained satisfied results were amendable to an overall test of the system functions. According to well-separated peaks in the chromatograms and data obtained for the retention times of diluent, standard and samples, it was concluded that no peaks were observed at the retention time of the ethanol. The linearity of the method was confirmed over the entire concentration range from 1 % (LOQ) to 150 %. The calibration curves of all 7 marker solutions with appropriate concentration showed a good linear correlation coefficient  $r^2=0.9999$  within the test ranges. The recovery was found to be between 106.5 % and 118.9 %, calculated by standard addition method. Three levels: system repeatability, method precision and intermediate precision were used for checking parameter precision. Six standard and sample solutions were prepared individually as per the test method using single batches on different days on the same equipment. F-value was under 5.05 which indicates a very good precision of the method. The robustness of the method was measured following small changes in the three chromatographic conditions (column flow  $\pm 0.1$  mL/min and injector and detector temperatures  $\pm 5$  °C). The tailing factor and theoretical plate number were evaluated according to the calculated data (the tailing factor was below 1.5 and the theoretical plate number were greater than 10 000 for all variable conditions). Observing the effect of these changes through the value of the relative standard deviation, which was less than 15.0 %, proved that method was robust. LOD and LOQ parameters were calculated after noise determination, according to pharmacopeias and guidelines (3 x noise and 10 x noise respectively).

The benefit of this study is the development and validation of well – characterized and sensitive,

methodology, suitable for estimation of ethanol as a residual solvent in tablets.

## Conclusion

Ethanol as Class 3 residual solvent has no therapeutic benefits, it may be a hazard to human health so it must be ensured that it is either not present in the product or is only present below the recommended acceptable level. The development of the method was carried out according to ICH guidelines Q2 (R2), and excellent results were obtained within the accepted validation reference values. According to the method described in this paper and obtained result of the validation it can be concluded that the residual ethanol can be analyzed in tablets within the permitted limits. The developed method can be successfully applied for routine quality control for quantification of ethanol in the pharmaceutical product tablets which belongs to a class of medications benzodiazepine.

The validation procedure shows that the method is suitable for its intended purpose, and it is used in the formal stability life cycle analysis as well as routine analysis.

## References

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