Development and validation of an LC-MS/MS method for screening and quantification of trace N-nitrosamines in a pharmaceutical formulation

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Organic compounds such as N-nitrosamines can occur naturally or be formed in the environment and food. Many of those have been identified as DNA-reactive mutagens and could lead to cancer.

In recent years, significant amounts of N-nitrosamine impurities have also been found in pharmaceutical products for human use, raising serious health concerns.

Therefore, this work aimed to develop and validate a highly specific and sensitive analytical method coupling liquid chromatography with tandem mass spectrometry detection for the simultaneous screening and quantification of 5 trace impurities of N-nitrosamines. These N-nitrosamines were specifically selected in the context of the Quality Control of a commercialized pharmaceutical product.

The method was developed using an innovative *in-silico* approach combining the screening phase and optimization of the method allowing flexibility in terms of N-nitrosamines targeting and matrix challenges to be solved. Validation of the method was conducted under the recommendations of ICH Q2(R1) using the accuracy profile approach.

The present method is validated for the 5 N-nitrosamines both as a limit test for impurities and as an assay. This method demonstrates adequate quantitative performance. The validated dosing range is at least between 1 ng/mL and 30 ng/mL for all N-nitrosamines.