Simultaneous detection of unspecific trace N-nitrosamine impurities by LC-MS/MS in a pharmaceutical formulation

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N-nitrosamines, a class of organic compounds, can occur naturally or be formed in the environment and food. Many of these compounds are recognized as DNA-reactive mutagens with carcinogenic potential. In past years, significant levels of N-nitrosamine impurities have been found in pharmaceutical products for human use, prompting serious health concerns.

In response to these concerns, we developed a liquid chromatography method in reverse phase mode coupled with tandem mass spectrometry detection (LC-MS/MS) allowing versatile application, as well as high sensitivity and specificity. Our work aimed to validate generic LC-MS/MS methods for the simultaneous determination of various trace impurities of N-nitrosamines. We focused on those unrelated to the drug substance, specifically for the Quality Control of a commercial pharmaceutical product. The generic methods were designed to offer flexibility in application, requiring no modifications in sample preparation or equipment configuration and thus allowing a single-run (sequential) analysis of 17 N-nitrosamines.

Method validation was conducted according to the ICH Q2(R2) guidelines. Upon successful validation, these methods were subsequently used to determine unspecific N-nitrosamine content in the selected pharmaceutical formulation, as mandated by the European Medicines Agency for confirmatory testing.

The confirmatory testing results revealed the absence of certain N-nitrosamines, permitting the omission of their specification limits in routine analysis. However, one N-nitrosamine was detected, and its content was consistently determined below and around (for aged stability samples) the specification limit, necessitating routine quantitative impurity testing.