Simultaneous detection of small molecule 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 applications, as well as high sensitivity and specificity. Our work aimed to validate LC-MS/MS methods for simultaneously determining various N-nitrosamine impurities. We focused on small-molecule N-nitrosamines unrelated to the drug substance, specifically for the Quality Control of a commercial pharmaceutical product. The 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-nitrosamines content in the selected pharmaceutical product, as mandated by the European Medicines Agency for confirmatory testing from the “Call for review” investigation procedure.

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 (for aged stability samples) the specification limit, necessitating routine quantitative impurity testing.