In-silico-assisted development of LC-MS/MS methods for the determination of 17 N-nitrosamines in a drug matrix

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The presence of nitrosamine-type impurities in pharmaceutical products for human use is a significant risk to public health. Indeed, these compounds are recognized by regulatory health agencies as potentially or possibly carcinogenic to humans. Following multiple successive detections of N-nitrosamines in numerous classes of medicines such as sartans, anti-diabetics, antacids and antibiotics, this problem has now become worldwide and has attracted particular attention from the competent authorities. Therefore, the European Medicines Agency (EMA) and the Food and Drug Administration (FDA) have strongly recommended extending the experience gained to all medicines for human use. All the measures implemented aim to reassure patients that the medicines meet the requirements of safety, efficacy and quality.

N-Nitrosamines are usually generated via a nitrosation reaction between nitrites and secondary or tertiary amines, preferably at an acidic pH. Mostly, their formation is due to using a nitrosating agent in the synthesis or contaminated raw materials. However, N-nitrosamines can also be formed during storage, due to the degradation of active substances or even a reaction between active pharmaceutical ingredients (APIs) and packaging materials. Considering the potential risk of genotoxicity, EMA has established acceptable daily intake limits for certain N-nitrosamines and N-nitroso-APIs according to their toxicological profile. The specification limit of a given nitrosamine impurity expressed in ppm for a particular product is calculated by dividing its acceptable daily limit by the maximum daily dose of the medicinal product.

These impurities are typically present at trace levels in pharmaceutical products. A major challenge is to develop analytical methods capable of achieving extremely low detection and quantification limits. In general, chromatographic techniques coupled with mass spectrometry are used. Compared to gas chromatography, liquid chromatography (LC) offers a wider spectrum of analytes and is able to analyze both volatile and non-volatile nitrosamines.

This work highlighted the development of highly sensitive and specific methods coupling LC with tandem mass spectrometry (MS/MS). In the present work, an ACQUITY™ Premier system coupled to a Xevo™ TQ- Absolute system was used for the detection and quantification of N-nitrosamines under EMA investigation, such as N-nitrosodi-n-propylamine (NDPA), N-nitrosoethylisopropylamine (NEIPA), N-nitrosomorpholine (NMOR) and N-nitroso-N-methylaniline (NMPA), down to the order of 50 ppb in a selected drug matrix. The methods were developed by means of an innovative in-silico screening approach based on the concept of Quantitative Structure Retention Relationship (QSRR), to address the selectivity and matrix effect aspects of APIs and/or drug matrices at an early stage.