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Abstract

Nitrogen-containing organic compounds (NOCs) are a broad class of substances that includes, among others, amines, nitro compounds, *N*-nitrosamines (NAs), and nitrogen-containing heterocycles. Given the confirmed high toxicity and genotoxic potential of certain nitrogen-containing organic compounds—particularly *N*-nitrosamines—their presence in the human environment should be strictly monitored and regulated. In 2018, the detection of *N*-nitrosodimethylamine (NDMA) in batches of valsartan-containing medicines triggered global investigations and the introduction of new regulations concerning nitrosamine impurities. Since then, the development and validation of modern analytical methods for the determination of *N*-nitrosamines has assumed a key role in quality-assurance systems in pharmaceutical manufacturing and has become a foundation for assessing the safety of medicinal products. Detecting *N*-nitrosamines in various matrices at low concentration levels (ppb) remains a challenge for scientists worldwide. Risk assessments of manufacturing processes for active pharmaceutical ingredients (APIs) and medicinal products further indicate the possibility of the formation of new *N*-nitrosamines not yet described in the literature. The mechanisms underlying their formation during synthesis, formulation of dosage forms, and storage of APIs and medicinal products are still insufficiently understood, underscoring the need for further research in this area.

The aim of this work was to: perform a risk assessment for the formation of N-nitrosamines; develop sample-preparation approaches and innovative chromatographic methods for determining *N*-nitrosamines and/or nitrogen-containing organic compounds; validate the developed chromatographic methods; investigate the formation mechanisms of selected *N*-nitrosamines; and synthesize new nitrogen-containing organic compounds.

The work began with a risk assessment of *N*-nitrosamine formation in selected APIs to define the nature of the hazards associated with these impurities and to initiate actions aimed at controlling impurity profiles in medicinal products. Of key importance was the investigation of *N*-nitrosamine formation mechanisms in selected APIs, enabling the prediction and prevention of their occurrence by controlling precursors (NOCs), rather than focusing solely on the determination

of the resulting low-molecular-weight nitrosamines (NIs) or nitrosamine drug substance-related impurities (NDSRIs).

Experimental studies led to the development of two analytical methods for the determination and control of selected *N*-nitrosamines in APIs and medicinal products. First, a gas chromatography–mass spectrometry method was developed and validated for the simultaneous determination of nine genotoxic nitrosamines in three APIs—cilostazol, sunitinib, and olmesartan medoxomil—manufactured at the Łukasiewicz – Industrial Chemistry Institute (Ł-ICHP). Subsequently, as part of market surveillance, a liquid chromatography–tandem mass spectrometry method was validated and implemented to detect 1-methyl-4-nitrosopiperazine (MNP) in multicomponent preparations containing rifampicin, with explicit consideration of matrix effects. In addition, stability and degradation studies of rifampicin-containing medicinal products were carried out to better elucidate the processes leading to this undesired impurity.

The next stage involved the electrochemical generation of impurities in APIs containing a hydrazone group: dantrolene, nitrofurantoin, furazidine, and nitrofurazone. Use of the ROXY™ EC system enabled the investigation of oxidation and reduction processes of the selected APIs, followed by the identification of as many as 17 new impurities, supported by quantum-chemical calculations (DFT) and *in silico* analyses.

In summary, this work resulted in the development and implementation of modern, sensitive, and specific chromatographic methods coupled to mass spectrometry for the detection and quantification of N-nitrosamines and other nitrogen-containing organic compounds in medicinal products and APIs. The methods also enabled investigation of the mechanisms by which impurity profiles form in selected APIs and medicinal products. The implemented analytical methods not only address regulatory requirements but also contribute significantly to the advancement of knowledge on the mechanisms of formation of these compounds. Most importantly, they enhance the safety and quality of medicinal products on the Polish and European markets.

Keywords: *nitrogen-containing organic compounds, nitrosamines (NAs), chromatographic methods, mass spectrometry, quality control, nitrosamine formation mechanisms, pharmaceutical safety.*