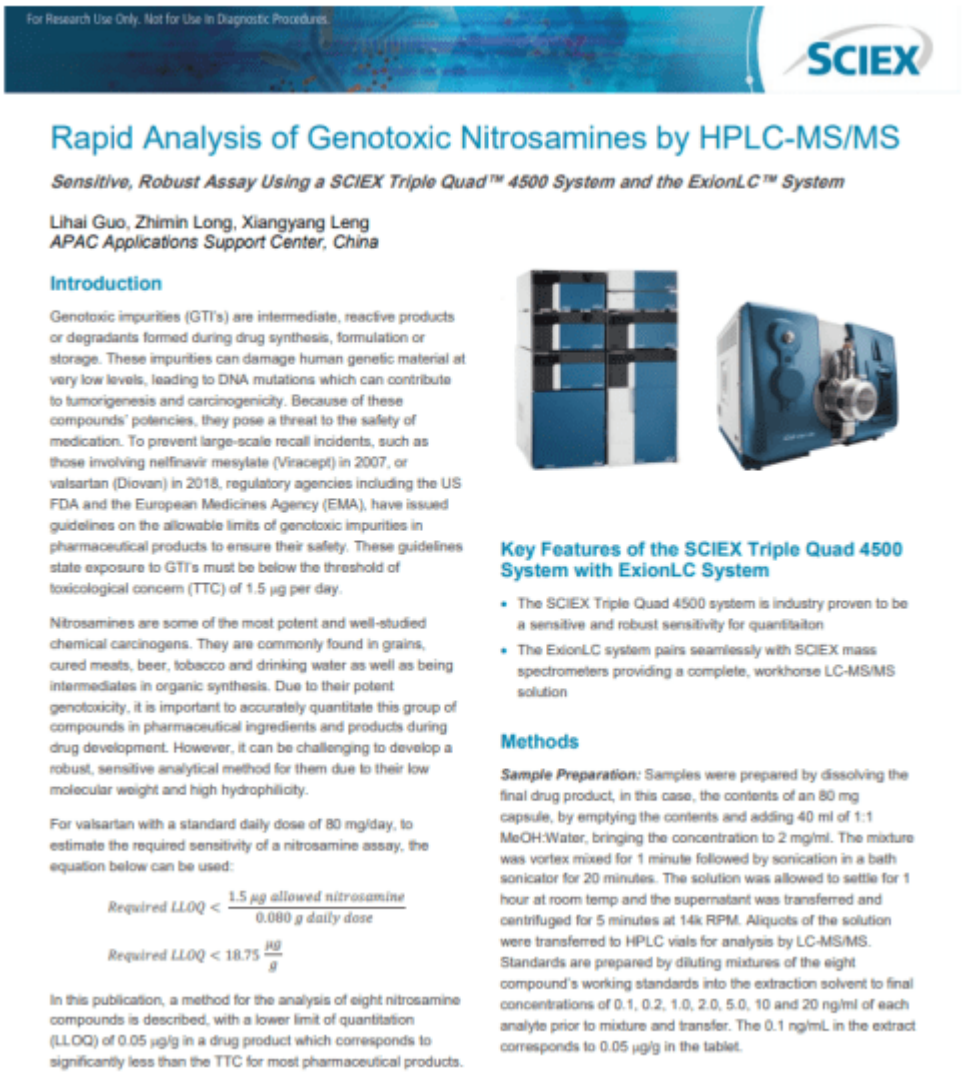
Guest Author: Phil Koerner, PhD.

The U.S. Food and Drug Administration (USFDA) and The European Medicines Agency (EMA) have set legally-binding limits for nitrosamine impurities in sartan blood pressure medicines. On their website, the EMA states that “companies must now take measures to avoid the presence of these impurities and carry out rigorous testing of their products.” This has increased the need for chromatographic testing methods, along with several LC-MS and GC-MS methods being published.

The SCIEX APAC Applications Support Center in Beijing, China has recently published an application note for the rapid analysis of eight genotoxic nitrosamines by HPLC-MS/MS with a reported LLOQ (lower limit of quantitation) of 0.1 ng/mL, equivalent to 0.05 µg/g of impurity in the drug product. This is lower than the threshold of toxicological concern defined by the EMA and USFDA. You can click the image below or the following link to see the full analysis: CLICK HERE



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SCIEX

Rapid Analysis of Genotoxic Nitrosamines by HPLC-MS/MS

Sensitive, Robust Assay Using a SCIEX Triple Quad™ 4500 System and the ExionLC™ System

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Introduction

Genotoxic impurities (GTI's) are intermediate, reactive products or degradants formed during drug synthesis, formulation or storage. These impurities can damage human genetic material at very low levels, leading to DNA mutations which can contribute to tumorigenesis and carcinogenicity. Because of these compounds' potencies, they pose a threat to the safety of medication. To prevent large-scale recall incidents, such as those involving nelfinavir mesylate (Viracept) in 2007, or valsartan (Diovan) in 2018, regulatory agencies including the US FDA and the European Medicines Agency (EMA), have issued guidelines on the allowable limits of genotoxic impurities in pharmaceutical products to ensure their safety. These guidelines state exposure to GTI's must be below the threshold of toxicological concern (TTC) of 1.5 µg per day.

Nitrosamines are some of the most potent and well-studied chemical carcinogens. They are commonly found in grains, cured meats, beer, tobacco and drinking water as well as being intermediates in organic synthesis. Due to their potent genotoxicity, it is important to accurately quantitate this group of compounds in pharmaceutical ingredients and products during drug development. However, it can be challenging to develop a robust, sensitive analytical method for them due to their low molecular weight and high hydrophilicity.

For valsartan with a standard daily dose of 80 mg/day, to estimate the required sensitivity of a nitrosamine assay, the equation below can be used:

$$Required\ LLOQ < \frac{1.5\ \mu g\ allowed\ nitrosamine}{0.080\ g\ daily\ dose}$$

$$Required\ LLOQ < 18.75\ \frac{\mu g}{g}$$

In this publication, a method for the analysis of eight nitrosamine compounds is described, with a lower limit of quantitation (LLOQ) of 0.05 µg/g in a drug product which corresponds to significantly less than the TTC for most pharmaceutical products.

Key Features of the SCIEX Triple Quad 4500 System with ExionLC System

- The SCIEX Triple Quad 4500 system is industry proven to be a sensitive and robust sensitivity for quantitaiton
- The ExionLC system pairs seamlessly with SCIEX mass spectrometers providing a complete, workhorse LC-MS/MS solution

Methods

Sample Preparation: Samples were prepared by dissolving the final drug product, in this case, the contents of an 80 mg capsule, by emptying the contents and adding 40 ml of 1:1 MeOH:Water, bringing the concentration to 2 mg/ml. The mixture was vortex mixed for 1 minute followed by sonication in a bath sonicator for 20 minutes. The solution was allowed to settle for 1 hour at room temp and the supernatant was transferred and centrifuged for 5 minutes at 14k RPM. Aliquots of the solution were transferred to HPLC vials for analysis by LC-MS/MS. Standards are prepared by diluting mixtures of the eight compound's working standards into the extraction solvent to final concentrations of 0.1, 0.2, 1.0, 2.0, 5.0, 10 and 20 ng/ml of each analyte prior to mixture and transfer. The 0.1 ng/mL in the extract corresponds to 0.05 µg/g in the tablet.

The EMA continues to closely monitor drug manufacturing, and as a precaution, has recently called on companies using certain reagents to manufacture pioglitazone, a diabetes drug, to test their products and check their processes to rule out the presence of nitrosamine impurities, in particular nitrosodimethylamine (NDMA). This follows the detection of low levels of NDMA in a few batches of pioglitazone manufactured by Hetero Labs in India.

In March of this year, Hetero announced a recall of 87 lots of losartan potassium tablets, which had been found to contain another nitrosamine impurity NMBA (N-Nitroso-N-methyl-4-aminobutyric acid). This impurity is a known animal and potential human carcinogen. Shortly after, Torrent and Teva recalled losartan in the US due to the presence of NMBA in the API manufactured by Hetero, which exceeded the USFDA's interim acceptable exposure limit of 9.82 ppm. More recently, another nitrosamine, N-Nitroso-N-methyl-4-aminobutyric acid (NMBA), was reported in losartan potassium tablets, resulting in the product also be recalled from the market.

These sartan drug medicine recalls have been necessary because the exposure to genotoxic nitrosamines poses an unacceptable safety risk to patients. However, it has resulted in a shortage of these drugs as well. To keep up with the latest news on the topic, you can visit the FDA website: CLICK HERE, or the EMA website: CLICK HERE

And if you are interested in a deeper dive into sartan drugs, check out our earlier article, "Sartan Drugs Have Been Deemed in Shortage by the FDA", which focuses on the make-up and how sartan drugs could affect you.

> Sartan Drugs Have Been Deemed in Shortage by the FDA

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