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Genotoxic Impurities

Genotoxic Impurities – A QC Laboratory Perspective

Over the past decade or so, the issue of impurities present in pharmaceuticals which may be DNA-reactive has caused regulators and manufacturers significant problems. This has been highlighted by the recall of various products throughout 2019 due to issues with NDMA.

Whilst there are tests for determining Carcinogenicity and Mutagenicity for safety labelling purposes, these generally lack the sensitivity to detect Genotoxic (i.e. the property of being able to damage cellular DNA and induce genetic mutations) impurities which are often only present at parts per million (ppm) levels.

Much has been written on the terminology, including the ICH guidance on Genotoxicity testing (ICH S2) and the responsibility of drug manufacturers to identify potential sources of impurities to be tested for and reported upon.

However, there do not appear to be many publications covering the subject of laboratory testing for such materials other than the broad approaches of using GC-MS and LC-MS etc. This is probably not surprising as companies who develop methodologies will consider such information confidential.

Analysis of Genotoxic Impurities (GTIs) is challenging as the levels of impurities must be reduced to acceptable safety limits to protect patients and therefore the analytical methods need to reach down to a range of 1 – 5ppm or lower.

Whilst sensitivity of the instruments is a major factor, the presence of other organic impurities at similar levels along with relatively high concentrations of active ingredients means that highly specific methods need to be carefully designed. Added to this, by their very reactive nature, many GTIs can easily react during their extraction and sample preparation as well as during final analysis which can lead to inaccurate and/or variable results.

Some identified GTIs are volatile in nature and one common technique applied is Headspace GC-MS. Commonly used in QC laboratories for residual solvent analysis, it has been applied to GTIs such as halides, sulfonates and epoxides. In 2016, five new General Chapters on Genotoxic Impurities in Pharmaceutical APIs were introduced into the European Pharmacopoeia:

- 2.5.37 Methyl, ethyl and isopropyl methanesulfonate in methanesulfonic acid
- 2.5.38 Methyl, ethyl and isopropyl methanesulfonate in active substances
- 2.5.39 Methanesulfonyl chloride in methanesulfonic acid

- 2.5.40 Methyl, ethyl and isopropyl toluenesulfonate in active substances
- 2.5.41 Methyl, ethyl and isopropyl benzenesulfonate in active substances

All of the above methods are based on Headspace GC-MS and, being less specific in the detail, listed in the General Chapters rather than specific material monographs. Butterworths, as a contract laboratory with extensive experience of headspace analysis, has been able to adapt the principle of the methods to develop and validate methods for:

- Alkyl halides, Residual Toluenesulfonates (Tosylates) and Residual Hydrazine in a non-ergo-derived selective dopamine D2 receptor
- residual Methylsulfonates (Mesylates) in a melanoma skin cancer drug
- DMAP (4-dimethylaminopyridine) in a female hormone.

For non-volatile GTIs, HPLC is often the first choice technique due to its availability in most QC laboratories and its ease of use.

UPLC is also often used with its shorter run times resulting from smaller particle size columns. However, where available, the use of LC-MS generally provides greater selectivity, robustness and ruggedness in addition to lower detection limits.

Our triple-quadrupole mass spectrometer works well for the confirmation of known impurities and the preliminary structural assessment of unknown impurities.

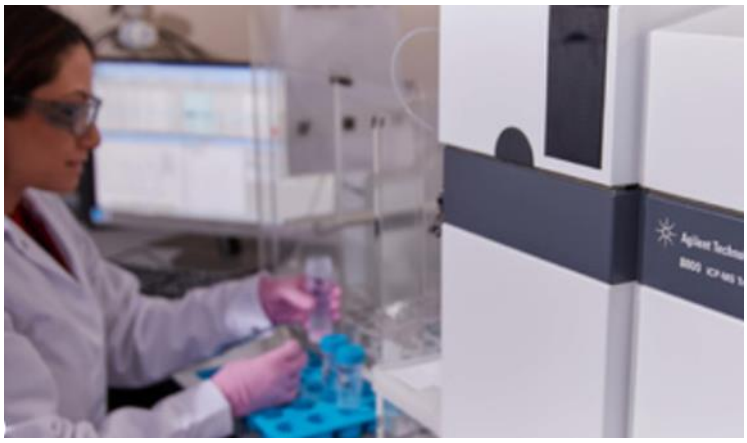
European Pharmacopoeia monographs of valsartan, candesartan, irbesartan, losartan, and olmesartan have just been revised and published on 1st January, 2020 with limits set for for N-nitrosodimethylamine (NDMA) and N-nitrosodiethylamine (NDEA) (see Table below).

Similar interim specification limits were set by the Food and Drug Administration (FDA). Products containing NDMA or NDEA above these limits or containing both impurities at any level will have to be withdrawn from the market. After the transition period of 2 years, a strict limit of < 0.03 ppm for NDMA and NDEA will apply. Other nitrosamines which may be potentially present in “sartans” are e.g. N-nitrosoethylisopropylamine (NEIPA), N-nitrodiisopropylamine (NDIPA), N-nitrosodibutylamine (NDBA) and N-nitroso-N-methyl-4-aminobutanoic acid (NMBA).

API name	Maximum Daily Dose (mg/day)		NDMA interim limits (ppm)		NDEA interim limits (ppm)	
	EMA	FDA	EMA	FDA	EMA	FDA
Valsartan	320	320	0.300	0.3	0.082	0.083
Losartan	150	100	0.640	0.96	0.177	0.27
Olmesartan	40	40	2.400	2.4	0.663	0.66
Irbesartan	300	300	0.320	0.32	0.088	0.088
Candesartan	32	32	3.000	3.0	0.820	0.83

The first project undertaken by Butterworth Laboratories after commissioning their LC-MS instrument will be to evaluate and proceduralise a method for the analysis of NDMA and NDEA in Sartans based on published

FDA procedures, extending work already carried out using GC-MS to enable us to undertake testing of these materials. However, it should be noted that a method for the sample preparation for each client product will need to be validated to meet the EP Monograph requirements.



Certain metal impurities can also cause DNA mutations, with the most commonly used analytical techniques being ICP-OES and ICP-MS, in line with the EP General Chapter 2.4.20 or USP <232>/<233> for elemental impurities. With either technique, sample preparation can be by solubilisation in an aqueous or organic solvent, or more commonly after acid digestion using a closed-vessel microwave system.

It is clear that the General Chapters published in the EP with respect to GTIs, together with the General Chapters and Monographs on Impurities Control in general provide an excellent basis for developing routine test methods for the development of QC methods for GTIs once they have been identified as being present as part of the drug development process.

Whilst it is important that final products are screened to show the absence of, for example, Nitrosamines in the tablets, various EMA and FDA publications, point out that the source is often suspected to be one of the raw materials. Therefore, contract testing laboratories who specialise in raw materials testing, such as Butterworths, are an ideal solution for this testing requirement.

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John started at Butterworth in 1987 as an Analytical Chemist and has had various roles including Quality Assurance Manager and Business Development Manager before becoming Associate Director of Business Operations in 2018.

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