PEER REVIEWED

There's More to Achieving High Quality Results than a Top of the Line LC-MS (Although it Does Help)

Introduction

In offering the most sensitive and selective analysis, liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a cornerstone of modern pharmaceutical research. In pharmaceutical analysis, accuracy and consistency of results is essential in maintaining product quality, and as instrumentation becomes ever more sensitive, every material used in the analysis needs to feed into ensuring the best possible results. Whether this is the solvent, the high-performance liquid chromatography (HPLC) column or the gas supplied to the mass selective detector (MSD), every component of analysis can potentially impact on the quality of results through matrix effects and ion suppression[1].

Matrix effect can be defined as the combined effect of all components in a sample other than the analyte(s) of interest[2]. Depending on the method of sample preparation and the origin of the sample itself, background matrix will have a greater or lesser impact on an analyst's ability to reliably and repeatably detect compound(s) of interest[3]. Ion suppression is one form of matrix effect, the origin of which is not always easy to determine. Although the mechanisms of ion suppression are not always clear, impurities in the gas supplied to the MS is one effect that can cause reduction of signal to noise (S/N) ratio, which can mean that an LC-MS system will not provide repeatable results or, indeed, pass initial tests to check system performance. A comparison of multiple reaction monitoring (MRM) response to a standard compound such as the commonly used reserpine will provide S/N data that shows whether the system's sensitivity is within the expected specification.

By comparing the analyte signal in matrix compared to a standard in pure solvent, it is possible to see whether there are interferences that cause ion suppression. Again, this may show that ion suppression is occurring, but will not necessarily enable the analyst to pinpoint the source of the impurity that is affecting performance[1].

In many scenarios, instrument gas supply can be the source of interferences and background noise. Pharmaceutical labs tend to use either nitrogen gas supplied in bulk from multiple cylinders, from a liquid nitrogen dewar or supplied by a nitrogen generator. Nitrogen generators, such as Peak's Genius XE, remove many of the hassles of bulk nitrogen supply including frequent reordering and managing of stock levels and deliveries. With a generator, labs also don't need to worry about interruptions to supply, and can avoid the risk of running out of gas overnight, as well as the additional inconvenience of changing cylinders or bulk tanks when they have run out. Furthermore, with a generator, labs can remove the unpredictability of gas prices that can rapidly fluctuate and reduce health and safety concerns for lab workers having to move heavy cylinders around the lab, not to mention the danger of having large quantities of liquid N2 in the lab, which has the potential to rapidly alter the lab atmospheric composition, posing a risk to lab personnel in the event that ventilation is inadequate, or fails[6].

Genius XE generators were developed to offer market-leading purity of LC-MS grade nitrogen to ensure that laboratories can focus on their analysis, safe in the knowledge that the purity of gas provided by the supporting gas generator will provide the best possible results.

Genius XE generators use a nitrogen membrane for purification of the gas from compressed air. Membrane purification uses a hollow-fibre polymer bundle that separates out oxygen from incoming compressed air by allowing smaller O2 molecules to pass through the side walls of the fibres, while larger N2 molecules are retained within the membrane bundle and collect in a receiver tank. This method of N2 purification can produce high purity nitrogen at up to 99.5% at flow rates required for LC–MS applications (typically 15–70LPM, depending on the instrument).

What Are NMHCs and Why Are They Potentially an Issue?

Because the process of N2 purification relies on the use of ambient air, which is compressed by the internal oil-free compressors in the generator, any ambient impurities are a risk to the overall gas purity, since membrane purification may not purge impurities along with O2 because of their molecular size. Therefore, in some lab environments or without adequate filtration, additional compounds which are present in the air could be passed to the mass spectrometer,affecting sensitivity and also impacting on maintenance intervals.

Several small-chain hydrocarbons, including ethane, propane, butane and pentane, known as non-methane hydrocarbons (NMHC), are found in the atmosphere at a range of concentrations from parts per billion (ppb) to parts per million (ppm)[4], depending on time of year and geography[5]. NMHCs are known to play an important role in atmospheric chemistry and can pose a problem for researchers in laboratories, since they can cause interference with analytical results, depending on the nature of the analysis.

Developing an Innovative Solution

In recognition of the challenges of laboratory environments and the need to reduce the potential for gas quality to impact on results, all-new proprietary hydrocarbon removal technology was designed for Genius XE to provide labs a higher degree of protection from potential environmental contamination. Some labs will use in-line traps to reduce the effect of NMHC on analysis, but most of the options on the market have a very low capacity and do not offer a long-term solution to the challenges of laboratory environments, resulting in frequent filter changes and an increase in the cost of ownership.

The resulting filtration was developed to ensure a higher purity of nitrogen

than would otherwise be achievable by eliminating NMHC from ambient air. Testing at the National Physical Laboratory in the UK showed that Genius XE can reduce levels of NMHC to levels of <1ppm. The advanced NMHC filtration stage introduced in Genius XE affords you greater confidence in your results, especially when running higher sensitivity analysis or where presence of excessive NMHCs may interfere with target analytes.

Methods

Peak Scientific carried out comparison of the Genius XE and a generator lacking specific hydrocarbon removal technology to compare performance of the generator when checking signal to noise ratio during check-out of an Agilent 6495 Triple Quad Mass Spectrometer in a challenging lab environment.

Five replicates were run using each of two standards; chloramphenicol and reserpine. The 6495 Triple Quad Mass Spectrometer was operated in positive ion mode to test S/N of reserpine and in negative ion mode to assess S/N with chloramphenicol.

Results

Results showed a large difference in performance of the two generators when operating in positive ion mode. The S/N ratio of the chloramphenicol peak with the LC-MS running in negative ion mode shows that without HC removal technology, a dramatic decrease in S/N can be observed in some lab environments. An average of five injections showed that without hydrocarbon removal, the S/N was around 2200:1 compared with 93,000:1 with hydrocarbon removal in place.

When running a reserpine standard in positive ion mode, again a large difference in S/N was observed, with Genius XE's hydrocarbon removal technology giving an average S/N of 113,431:1 for five reserpine injections compared to 36,526:1 for samples run using a generator without hydrocarbon removal.

Discussion

Although lab infrastructure and processes are constantly improving, the effect of the environment and changes in airborne impurities is still beyond the control of many lab air conditioning systems. Seasonal fluctuations resulting in changing temperature and precipitation can change levels of airborne pollutants[4], particularly in large cities[5] which can impact on lab air quality without any discernible changes necessarily being noticed by staff. Gas generators are one of a number of components that interact directly, or indirectly, with the sample or the detector through the processes involved in sample preparation and analysis and can have an effect on results. Other components that have been identified as potentially introducing matrix into samples include solvents, which should always be selected carefully to avoid interaction with ionisation conditions[1], and tubing used to supply gas to the mass spectrometer. If poor quality plastic tubing is used that outgasses large quantities of plasticisers, this can cause matrix effects, in terms of masking target analytes, and can directly affect instrument performance as a consequence of outgassing volatiles causing ion suppression. If metal tubing is used, stainless steel or lab-grade copper tubing should be used, with epoxy resins to seal joints being avoided.

Recognition of the importance of every part of the process in obtaining the best results means that as analytical instruments improve, there is a need for all of the peripheral products, including gas generators, to keep up, which has been a driver for Peak's new product development and introduction of the new technologies in Genius XE. The results of this analysis show the importance of the hydrocarbon removal technology employed in Genius XE nitrogen generators in reducing ion suppression and demonstrate how LC-MS performance can be enhanced by using this state of the art nitrogen generator.

Standard tested	Chloramphenicol (-ve mode)		Reserpine (+ve mode)	
Gas source	Generator without HC removal	Genius XE70	Generator without HC removal	Genius XE70
Replicate 1 S/N	2178	99927	49139	118262
Replicate 2 S/N	2039	76943	26706	134984
Replicate 3 S/N	1532	126961	25523	115225
Replicate 4 S/N	2566	80180	37926	110140
Replicate 5 S/N	2578	83001	43334	88546
Average S/N	**2179**	**93402**	**36526**	**113431**

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