Carrier gas conservation for nitrosamines analysis in pharmaceutical products

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Introduction

Nitrosamines are ubiquitous compounds classified as probable carcinogens by the International Agency for Cancer Research (IARC). They, therefore, represent a matter of concern, especially after June 2018 when *N*-nitrosodimethylamine (NDMA) was reported in valsartan and several batches had to be recalled. Contamination of products can easily occur as small amounts of nitrosamines can be found in raw materials, reagents, and solvents used during the production processes, as well as in laboratory equipment, plastic and rubber materials, pipettes, and even nitrile gloves. The analysis of nitrosamines as an impurity in pharmaceutical products can be accomplished with high sensitivity and selectivity using gas chromatography-mass spectrometry (GC-MS).

Helium is the most used carrier gas for gas chromatography thanks to its high chromatographic efficiency and inertness. The helium shortage 4.0, which started in July 2021 and lead to dramatic price increases and delayed supply, pushed GC manufacturers, researchers, and analysts to evaluate alternative options such as the adoption of different carrier gases or, when not possible, the reduction of the helium gas consumption. The Thermo Scientific™ HeSaver-H_2Safer™ carrier gas saving technology[1] offers an innovative and effective approach to dramatically reduce helium consumption. It consists of a modified SSL injector body connected to two gas lines: an inexpensive gas (e.g., nitrogen or argon) is used for inlet pressurization, analyte vaporization,

and transfer to the analytical column, whereas the selected carrier gas (e.g., helium or hydrogen) is used only to feed the chromatographic column for the separation process. The carrier gas is therefore supplied with a maximum flow rate limited to few mL/min, thus reducing its overall consumption. When used with helium, the HeSaver-H_2Safer option allows GC-MS analytical performance to be preserved without the need of instrument method re-optimization otherwise required in case of migration to a different carrier gas.

A Thermo Scientific™ TRACE™ 1610 gas chromatograph equipped with two Thermo Scientific™ iConnect™ split/splitless injectors, one operating as a standard SSL and the other one upgraded to work in HeSaver-H_2Safer mode, was coupled to a Thermo Scientific™ TSQ™ 9610 triple quadrupole mass spectrometer and used to run a side-by-side comparison to demonstrate equivalency of chromatographic separation, linearity, and instrument detection limits (IDLs). A complete list of reagents, consumables, and instrument settings are reported in a previous application note.[2] For this evaluation, the TSQ 9610 triple quadrupole mass spectrometer was operated using an emission current of 10 µA.

Results and discussion

One of the key benefits of the HeSaver-H_2Safer inlet is that method transfer (for methods using the standard SSL injector) was straightforward and there was no need for re-optimization. The transition from the pressurization gas (nitrogen) to the carrier gas (helium) after the sample injection and transfer into the column, is extremely fast (few milliseconds), thus ensuring a rapid gas replacement without impacting on the chromatography and on the mass spectrometer performance. This was confirmed from the results obtained in these experiments.

The chromatographic separation remained unchanged with retention time (RT) consistency (deviation of ≤ 0.06 minutes) and Gaussian peak shapes (asymmetry factor, A_s ≤ 1.1) confirming the efficient transfer of the analytes, including NDMA, which is particularly challenging due to its polarity (Figure 1). Moreover, the mean accuracy (as % recovery on triplicate injections at 200 fg on-column) was within 77–102% for the investigated nitrosamines (Figure 1).

iConnect Inlet	NDMA	NMEA	NDEA	NIPEA	NDIPA	NDPA	NPYR	NPIP	NDBA
Retention time (min)									
SSL	4.59	5.21	5.7	6.14	6.53	6.94	7.37	7.51	8.27
He-Saver	4.53	5.16	5.67	6.12	6.51	6.92	7.35	7.5	8.25
Deviation (min)	**0.06**	**0.05**	**0.03**	**0.02**	**0.02**	**0.02**	**0.02**	**0.01**	**0.02**
Asymmetry (A_s)									
SSL	1.2	1.4	1	1.1	1	0.8	0.9	1.1	1
He-Saver	0.9	1.1	1.1	0.9	0.9	1	0.9	1.1	0.9
Mean accuracy (%)									
SSL	94	86	93	86	74	81	98	81	77
He-Saver	102	81	87	77	78	78	81	90	83

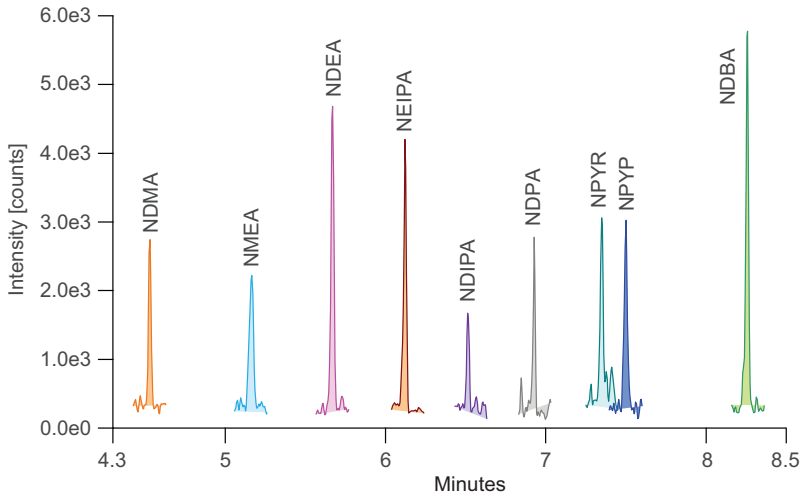


Figure 1. Chromatographic performance (as RT deviation, A_s, and % recovery) as well as an example of an extracted ion chromatogram (XIC) for a solvent standard at 200 fg on-column obtained by using the HeSaver-H_2Safer injector

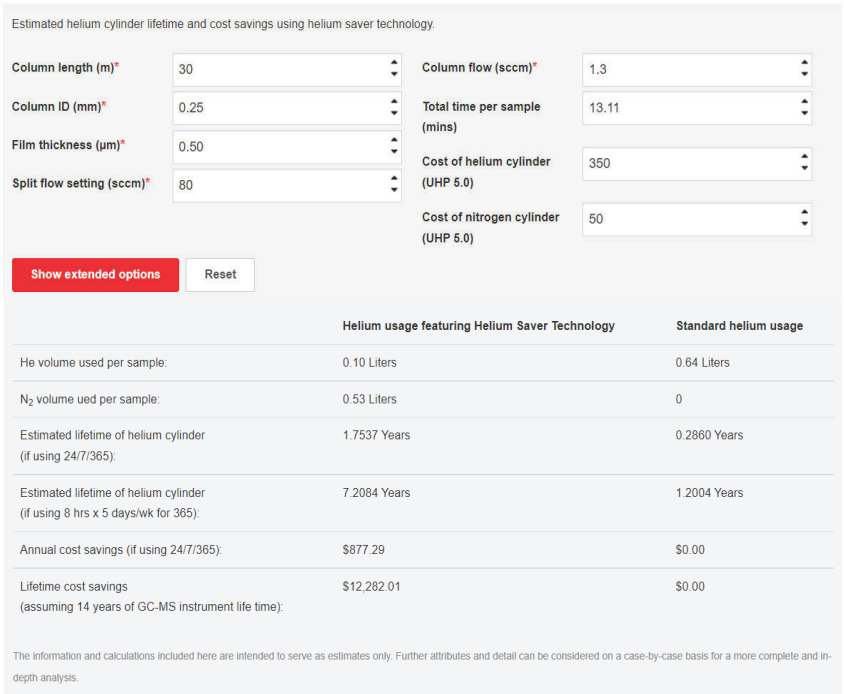
Linearity (expressed as coefficient of determination, R^2, and residual values measured as % RSD of average response factors, AvCF %RSD, for a calibration curve ranging from 0.1 to 5,000 ng/mL), sensitivity (expressed as IDLs calculated by injecting 200 fg on-column, n=10), and repeatability (expressed as absolute peak area repeatability calculated by injecting 200 fg on-column, n=10) showed analogous results when compared to the standard SSL injector (Table 1).

Reduced helium consumption and cost savings

The HeSaver-H_2Safer technology offers significant gas savings not only when the GC is idle, but mainly during sample injection and analysis. When used with helium as carrier gas, it translates into an extended helium cylinder lifetime from months to years, depending on the instrument method and usage, and how many GCs are connected. The Thermo Scientific™ Gas Saver Calculator tool[3] offers an easy-to-use and intuitive interface to estimate the helium consumption and cost impact. In a few clicks, the tool provides an estimation of both the helium cylinder lifetime and the cost savings based on the column geometry, the carrier and split flow settings, and the helium and nitrogen costs. The usage of the HeSaver-H_2Safer technology for the analysis of nitrosamines according to the U.S. FDA guidelines[4] would allow the helium cylinder to last about 6 times longer in comparison to the use of a standard SSL injector (Figure 2).

Table 1. Calculated R^2, AvCF %RSD, IDLs, and absolute peak area %RSDs obtained by comparing the HeSaver-H_2Safer technology and standard SSL injector

iConnect Inlet	NDMA	NMEA	NDEA	NIPEA	NDIPA	NDPA	NPYR	NPIP	NDBA
R^2									
SSL	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
He-Saver	0.998	0.999	0.999	0.999	0.999	0.999	0.999	0.999	0.999
AvCF %RSD									
SSL	7.2	6.1	6.2	7.6	6.3	7.6	6.6	7.8	4.9
He-Saver	9.3	8.2	5	5.8	6	6.5	4.9	6.5	4.7
IDL (ng/mL)									
SSL	0.03	0.02	0.02	0.02	0.03	0.03	0.04	0.03	0.03
He-Saver	0.02	0.03	0.02	0.03	0.02	0.03	0.03	0.03	0.03
Absolute peak area %RSD									
SSL	9.8	7.3	7	7.3	12.6	12.3	14.5	12.3	9.4
He-Saver	8.9	9.7	7.8	9.4	8.2	11.5	12.5	11.4	9.7



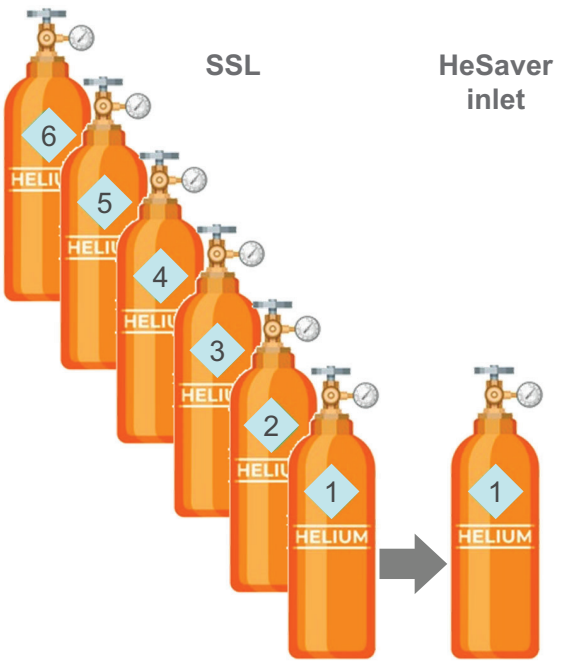


Figure 2. Gas Saver Calculator reporting the helium saving according to the U.S. FDA guidelines. The cylinders cost is indicative and country dependent.

Summary

The use of the HeSaver-H_2Safer option offers the advantage of reduced helium gas consumption without compromising GC-MS performance through a smooth and simple upgrade of a standard iConnect SSL injector module.

- Existing validated methods can be used unchanged, with consistent analytical performance in terms of injection repeatability, analyte transfer, recovery, linearity, and sensitivity. The fast transition when swapping from inexpensive pressurizing gas (injection phase) to the best carrier gas (separation process) ensures efficient transfer of analytes to the analytical column without impacting on retention times and peak shapes and preserving GC-MS analytical performance.
- An additional benefit of using two different gases for inlet pressurization and the separation process is that the pressurizing gas is discharged only through the split line for most of the time, entering the column just for the limited time of the injection phase, thus limiting the transfer of possible contaminants and matrix high boilers.
- The Helium Saver Calculator tool allows for easy and immediate estimation of helium cylinder lifetime and cost savings when using the Helium Saver technology.

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