Reduction of helium usage for the analysis of pesticide residues in botanical dietary supplements using GC-MS/MS with the HeSaver-H_2Safer technology

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Introduction

Consumer interest in botanicals has grown considerably over the past few years as they are considered to contribute to overall health and wellbeing. Supplements are the most popular botanical products sold over the counter in pharmacies, groceries and online stores, therefore, manufacturers must ensure that the raw materials are safe for consumption. Pesticides are chemicals used for crop protection against a variety of pests such as weeds, fungi, rodents, and insects. Because of their extensive use, pesticides can be found in the air, soil, water, and ultimately in the food chain. Despite their use being highly regulated, misuse of pesticides can lead to unwanted contamination of food or other plant-based consumer products, and have possible impacts on both human and environmental health.

When analyzing pesticides, GC-MS/MS is employed to detect trace levels. Helium is the most common carrier gas used for gas chromatography thanks to its high chromatographic efficiency and inertness. Recent price rises in helium and supply issues caused by shortages have led GC manufacturers, researchers, and analysts to investigate possible mitigation options that entail either switching to alternative carrier gases or reducing the helium consumption. The Thermo Scientific™ HeSaver-H_2Safer™ carrier gas saving technology[1] offers an innovative and smart approach to dramatically reduce carrier gas consumption, especially during GC operation. It consists of a modified SSL body connected to two gas lines: whereas an inexpensive gas (e.g., nitrogen or argon) is used for inlet pressurization, analyte vaporization and transfer to the analytical column, the selected carrier gas (e.g., helium or hydrogen) is used only to supply the chromatographic column for the separation process, with a limited maximum flow rate. When used with helium as carrier gas, the limited consumption helps to mitigate shortage issues while maintaining GC-MS performance without the need for instrument method re-optimization otherwise required in case of migration to a different carrier gas.

In this analysis 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 using argon as pressurizing gas, was coupled to the Thermo Scientific™ TSQ™ 9610 triple quadrupole mass spectrometer and used to run a direct comparison to demonstrate consistency in the analytical results. Details regarding the standards, reagents and the sample preparation used in this study are reported in a previous application note.[2]

Results and discussion

One of the key benefits of the HeSaver-H_2Safer inlet is that the method developed for the standard SSL injector was directly transferred to the HeSaver-H_2Safer injector without the need for re-optimization, as the chromatographic performance remained unchanged with retention time (RT) consistency (average deviation of 0.05 minutes) compared to standard SSL. Efficient transfer of analytes was demonstrated by the Gaussian peak shapes observed in the extracted ion chromatograms (XICs) of Senna tea extract spiked with a pesticide mix at 50 µg/kg (or ppb) (Figure 1).

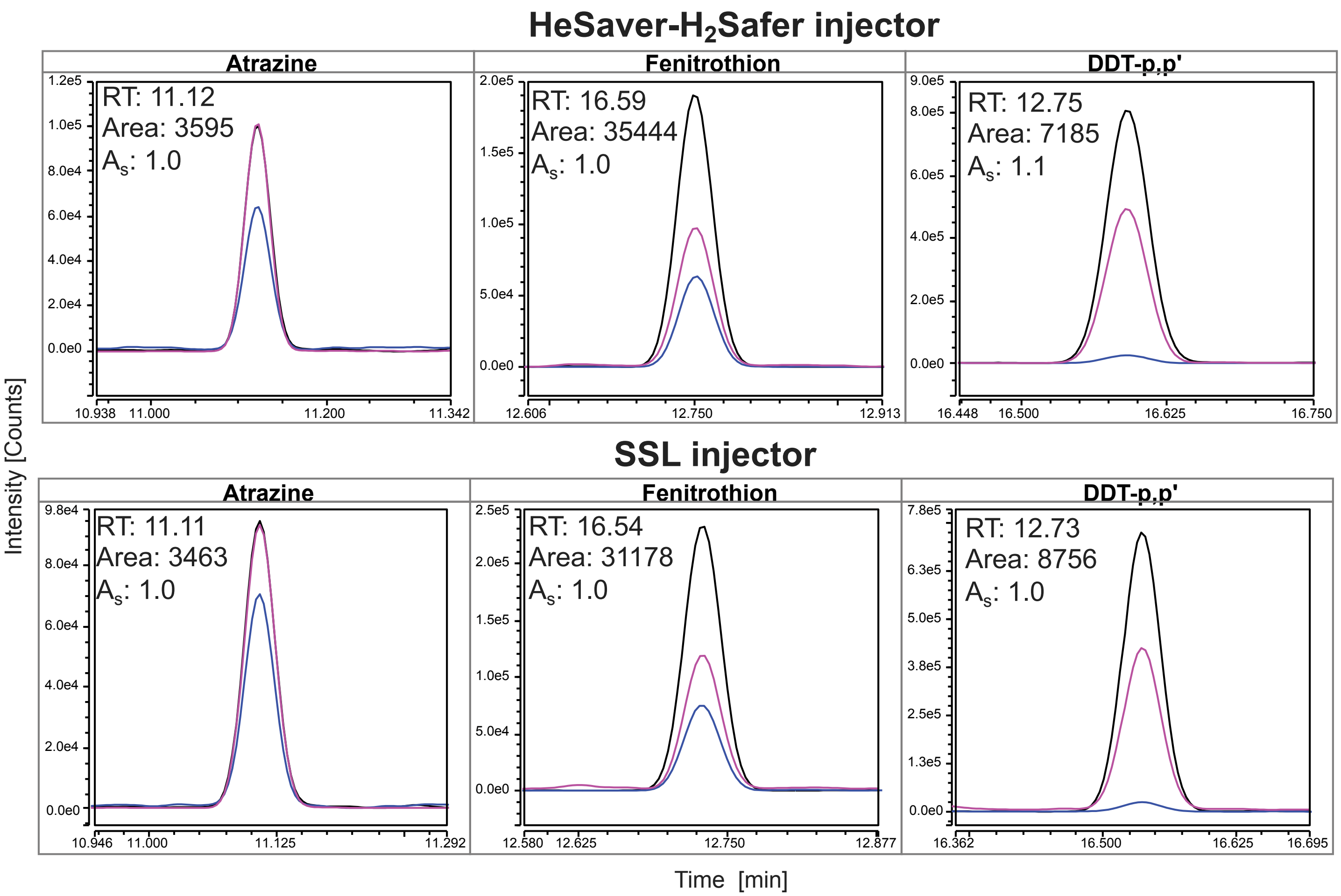

Figure 1. Examples of the chromatographic performance achieved with the HeSaver-H_2Safer and standard SSL inlets for a Senna tea extract spiked with a pesticide mix at 50 µg/kg. Peak area and asymmetry (As) values are annotated.

A five-point calibration curve ranging from 0.0 (un-spiked matrix) to 20 µg/kg was built around the action limit of 10 µg/kg in order to make sure that if any residue is detected, its quantitation would be accurate. Calculated coefficient of determination (R^2) as well as residual values (measured as %RSD of average response factors, AvCF %RSD), showed similar results when compared to the SSL injector, demonstrating that the method validated with standard SSL, can be applied without any modification to the HeSaver-H_2Safer inlet. As an example, calibration curves for some selected compounds obtained using the standards SSL and the HeSaver-H_2Safer injectors are shown in Figure 2.

Botanical supplements are sold in capsules, tablets or tea bags, making the analysis of pesticides even more challenging due to the low water content of the samples and to the high concentration of co-extracts that can be generated during the drying and manufacturing processes. The HeSaver-H_2Safer concept of decoupling the inlet pressurizing gas and the carrier gas reduces contamination from the injector into the column: the pressuring gas flushing the liner and the injector is discharged only through the split line for most of the time, entering the column just for the limited time of the injection phase, therefore, limiting the transfer of contaminants (septum/sample matrix/by-products). This was evaluated by pre-spiking (with a pesticide mix at 5 µg/kg) and extracting different botanicals (Guggel, Mukuna, Kava Kava root, Rehmannia root, Yerba Mate, Cayenne, Turmeric, and Senna tea) which provided clean extracts (e.g., Guggel and Mucuna) as well as challenging extracts (e.g., Kava Kava, Turmeric). An example of XICs for some selected compounds in Mucuna (clean matrix) and Kava Kava (complex matrix) spiked with a pesticide mix at 5 µg/kg is shown in Figure 3. No inlet, MS maintenance or re-tuning were required during the method evaluation with multiple matrices despite the injection of dirty matrix samples such Kava Kava root and Turmeric.

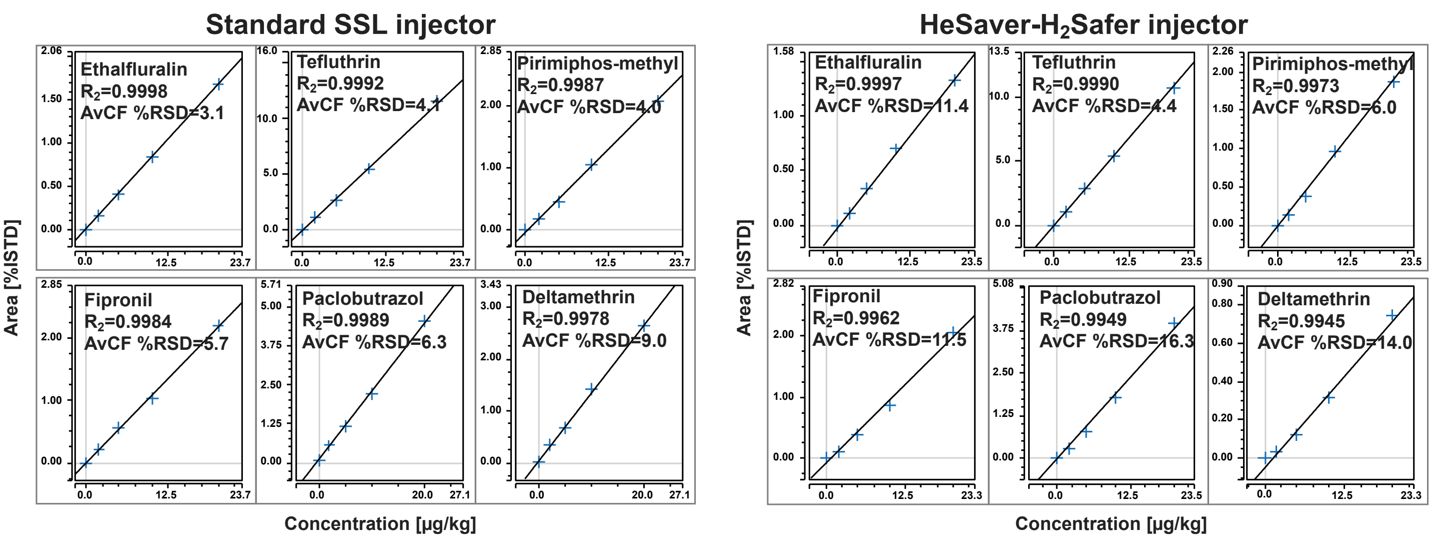

Figure 2. Examples of calibration curves for some selected compounds obtained using the standards SSL and the HeSaver-H_2Safer injectors. Calculated coefficient of determination (R^2) and residual values (measured as % RSD of average response factors, AvCF %RSD) are annotated.

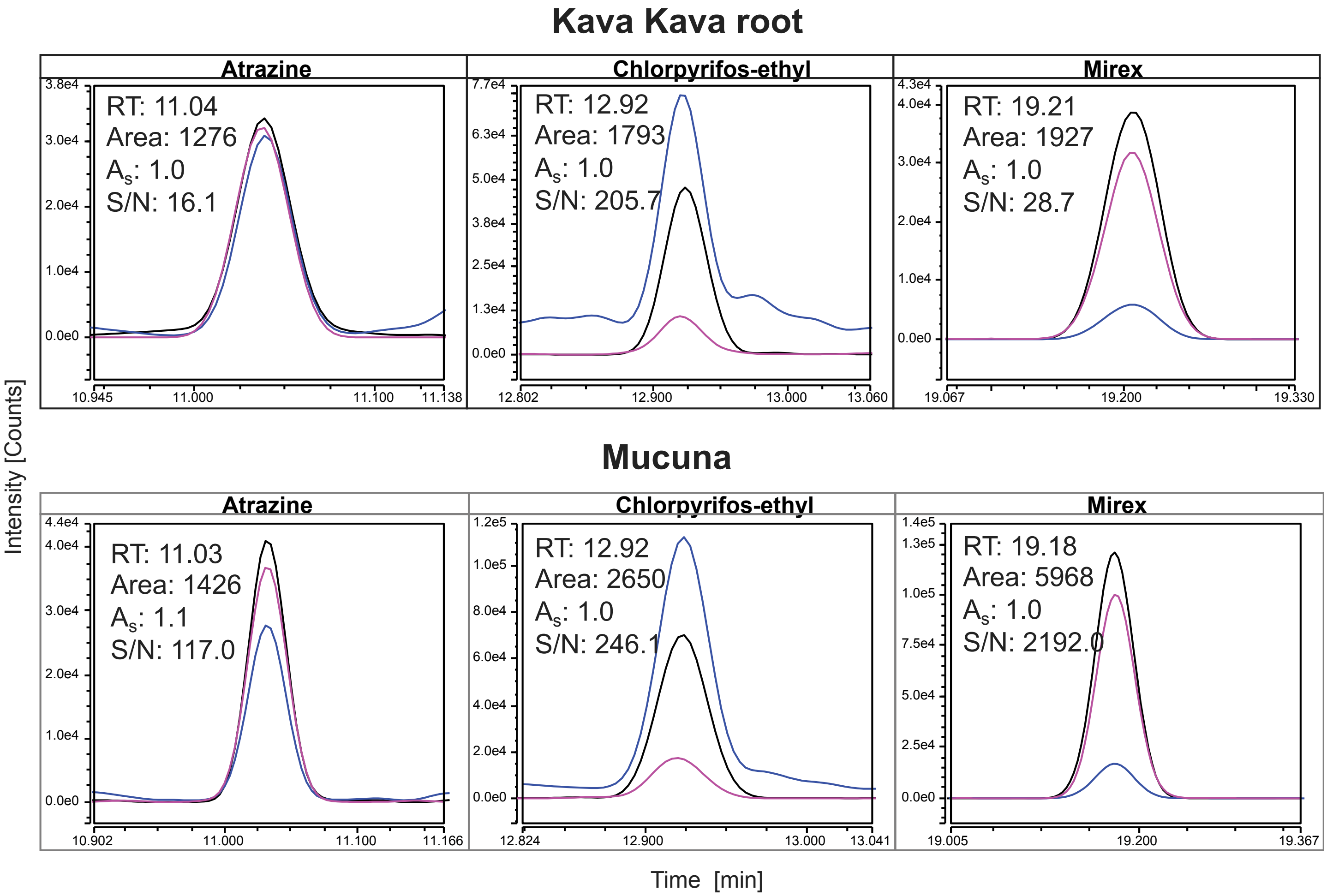

Figure 3. Example of XICs for atrazine, chloripyrifos-ethyl and mirex in Mucuna (clean matrix) and Kava Kava (complex matrix) extracts pre-spiked with a pesticide mix at 5 µg/kg.

Accuracy and precision (in terms of % recovery) of the HeSaver-H_2Safer inlet was evaluated by extracting and analyzing six different matrices (Guggel, Mukuna, Kava Kava root, Rehmannia root, Yerba Mate, and Cayenne) pre-spiked with a mix of pesticides at 10 and 50 µg/kg. Excellent accuracy and precision were obtained with calculated amount within 25% for the low spiked concentration and 10% for the high spiked concentration, and recovery between 78 and 97%. An example for some selected compounds is reported in Table 1. This demonstrates that the transition from the injection phase using an inexpensive pressurizing gas to the separation process using the best carrier gas is extremely fast (within a few milliseconds), thus, ensuring efficient transfer of the analytes from the inlet to the column with consistent separation efficiency and sensitivity compared to the use of a standard SSL injector.

Table 1. Accuracy and precision (in terms of % recovery) of the HeSaver-H_2Safer inlet evaluated by extracting and analyzing six different matrices pre-spiked with a mix of pesticides at 10 and 50 µg/kg.

Precision and spike recovery (n=6)*				
Compounds	Concentration %RSD	Average %Recovery	Concentration %RSD	Average %Recovery
	10 µg/kg Spike	10 µg/kg Spike	50 µg/kg Spike	50 µg/kg Spike
Atrazine	19.8	85	9.6	**97**
Chlorpyrifosethyl	24.4	90	7.9	**89**
DTT-p,p’	22.6	79	9.7	**81**

* n = 6 refers to recovery values obtained of each pesticide from six different sample matrixes.

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. The user is only required to insert the column geometry, the carrier and split flow settings, as well as helium and nitrogen costs, and the tool provides an estimation of both the helium cylinder lifetime and the cost saving. The usage of the HeSaver-H_2Safer technology for the analysis of pesticides would allow the helium cylinder to potentially last about 4 times longer in comparison to the usage of a standard SSL injector (Figure 4), making the helium-saver technology an ideal choice for helium gas saving.

Without helium-saver technology on their GC-MS/MS system, NOW Foods, a manufacturer of natural health products and supplements based in Illinois, changes their helium cylinder every 4 to 6 months. By monitoring the usage of the system with the HeSaver-H_2Safer installed, helium consumption is approximately 173 psi per month. If a full helium cylinder has a total of 2,500 psi, projected lifetime of a helium cylinder would be around 14 months. This means in real-world usage the helium cylinders will last 2.5–3.5 times longer with the HeSaver-H_2Safer technology for the analysis of pesticides. This is a significant cost and resource saving.

Summary

The HeSaver-H_2Safer technology offers the advantage of nearly 4 times reduced helium gas consumption, without compromising GC-MS performance for the analysis of residual pesticides in botanical supplements, through a smooth and simple upgrade of a standard iConnect SSL injector module.

- The transition from the injection phase using an inexpensive pressurizing gas to the separation process using the best carrier gas is extremely fast (within a few milliseconds), ensuring a rapid gas replacement into the column and, thus, analogous performance compared to the use of a standard SSL injector.
- Existing validated methods can be used unchanged with consistent analytical performance in terms of injection repeatability, analyte transfer, linearity, recovery and robustness.
- Moreover, the HeSaver-H_2Safer technology provides additional advantages, such as discharging the pressurizing gas only through the split line for the majority of the time, protecting the column from possible contaminants.
- The Helium Gas Saver Calculator allows for easy and immediate estimation of helium cylinder lifetime and cost savings when using the helium-saver technology.

Estimated helium cylinder lifetime and cost savings using helium saver technology.

Column length (m)*	30	Column flow (sccm)*	1.4
Column ID (mm)*	0.25	Total time per sample (mins)	26.5
Film thickness (µm)*	0.25	Cost of helium cylinder (UHP 5.0)	350
Split flow setting (sccm)*	60	Cost of nitrogen cylinder (UHP 5.0)	50

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	Helium usage featuring Helium Saver Technology	Standard helium usage
He volume used per sample:	0.22 Liters	0.89 Liters
N_2 volume ued per sample:	0.67 Liters	0
Estimated lifetime of helium cylinder (if using 24/7/365):	1.6966 Years	0.4128 Years
Estimated lifetime of helium cylinder (if using 8 hrs x 5 days/wk for 365):	6.9794 Years	1.7292 Years
Annual cost savings (if using 24/7/365):	$549.62	$0.00
Lifetime cost savings (assuming 14 years of GC-MS instrument life time):	$7,694.71	$0.00

The information and calculations included here are intended to serve as estimates only. Further attributes and detail can be considered on a case-by-case basis for a more complete and in-depth analysis.

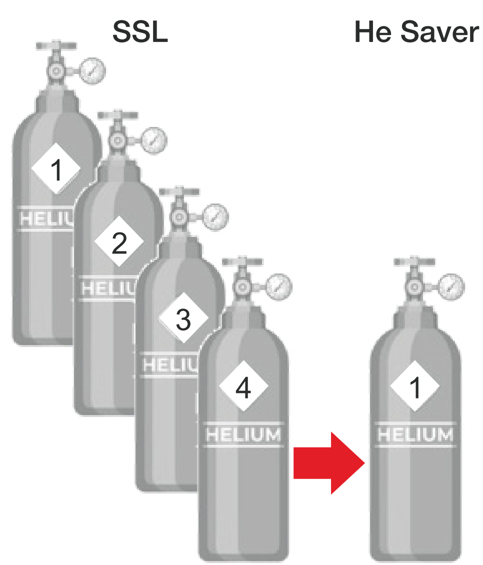

Figure 4. Gas Saver Calculator reporting the helium saving. The cylinders cost is indicative and country dependent.

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