Increased Reproducibility in the analysis of EU and EPA PAH's with the Agilent Select PAH GC Column and Metal Microfluidic Guard Chip Technology by Gas Chromatography

Abercrombie V, Anumol T, Serrano-Izaguirre G, Provoost L, Lee J

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Introduction

The analysis of Polycyclic Aromatic Hydrocarbons (PAH's) required by the European Union (EU) and the US Environmental Protection Agency (EPA) contains a combined list of 24 regulated compounds plus triphenylene, and can be chromatographically challenging. Even with good sample preparation, frequent maintenance, is needed to maintain peak shape, sensitivity, and avoid loss of signal. Frequently, liner replacement and column trims are needed to fully maintain the performance of the system.

The Agilent Intuvo 9000 uses enabling Metal Microfluidic Technology in the form of an easily replaceable guard chip, with an identical path length, as part of the inert flow path, eliminating the need to trim the column. By removing column trimming, less time is required to perform maintenance, and retention times are left unchanged. To evaluate the benefits of the Intuvo Guard Chip, we evaluate the analysis of PAH compounds in salmon oil.

PAH's in salmon oil may come from petrogenic or pyrogenic origin and can contaminate seafood due to accumulation of petroleum constituents in water sources. Since levels of concern for PAHs can be as low as 1 ng/mL, a robust and efficient of analysis method is required for accurate detection. pathlength it allows for consistent retention times after replacement.

Experimental

A commercially available salmon oil was prepared at a 1:10 dilution in Dichloromethane (Sigma) and analyzed on an Agilent 7890 GC and an Agilent Intuvo 9000 GC using an Agilent Select PAH GC Column. Standards of PAH compounds, at concentrations ranging from 1 ng/mL to 1000 ng/mL were injected every 50 injections of salmon oil, and column maintenance was performed to maintain sensitivity.

QuEChERS Enhanced Matrix Removal-Lipid (EMR) Sample preparation was also used to further clean up samples and maintain sensitivity, and increase time between the need for maintenance.

GC Conditions

Column	**Agilent Select PAH** 30m x 0.25 mm x 0.15 µm (p/n CP7462) 30m x 0.25 mm x 0.15 µm Intuvo Format (p/n CP7462-INT) 15m x 0.15 mm x 0.10 µm Intuvo Format (p/n CP7461–INT)
Carrier	**Helium, constant flow, 1.2 mL/min**
Oven	**70°C (0.7 min), Ramp 85°C/min to 180°C (0.0 min), Ramp 3°C/min to 230°C (10.0 min), Ramp 28°C/min to 280°C (20 min), Ramp 5°C/min to 330°C (5.0 min)**
Inlet	**Splitless mode, 300°C, Split to Vent 50 mL/min at 0.75 min**
Inlet Liner	**Ultra Inert, splitless, single taper, glass wool (p/n 5190-2293)**
GC/MSD	**Agilent 7890B GC and Intuvo 9000 equipped with Agilent 5977 MSD**
Sampler	**Agilent 7693 autosampler**

Results and Discussion

While the separation of the 25 PAH compounds can be challenging, Figure 1 demonstrates that resolution of isomers is possible with the Agilent Select PAH GC Column and mass spectral acquisition in Selective Ion Mode. High fat matrices, such as salmon oil, can be problematic if not prepared properly. Even a traditional dilution in a solvent such as DCM, can still lead to matrix accumulation in the flow path.

In Figure 2 a standard of 50 ng/mL is injected before and after 50 injections of salmon oil diluted 1:10 in dichloromethane. After the 50 injections of dilute salmon oil a dramatic loss in sensitivity is observed, as well as build up in the inlet liner, gold seal and septum. To regain sensitivity a front end maintenance was performed, replacement of inlet liner, gold seal, and septum and clipping a meter of column on the traditional 7890 GC. On the Intuvo 9000, the guard chip, a piece of metal microfluidic technology with identical path length, was replaced. Figure 3 demonstrates the resulting retention time shift on analytes as a result of front end maintenance on the 7890, versus no retention time shifting when replacing the guard chip on the Intuvo 9000. The ability to maintain retention times, increases the reproducibility of the analysis of PAH compounds in high fat matrices, such as salmon oil.

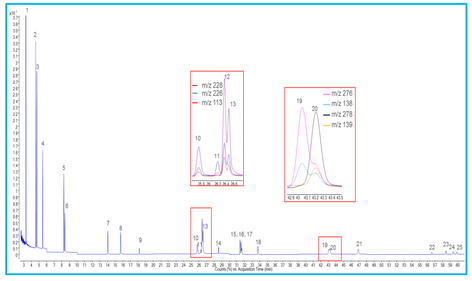

Figure 1. 50 ng/mL standard of 25 PAH compounds analyzed on a Agilent 7890 GC with a Agilent Select PAH GC column

MSD SIM Parameters

Peak	Component	CAS	SIM Group	m/z	Q1	Q2	EU 15+1	EPA
1	Naphthalene	91-20-3	1	128				x
2	Acenaphthylene	208-96-8		152				x
3	Acenaphthene	83-32-9		154	128	152		x
4	Flourene	86-73-7		166				x
5	Phenanthrene	85-01-8	2	178	76			x
6	Anthracene	120-12-7		178	76			x
7	Flouranthene	206-44-0	3	202	101			x
8	Pyrene	129-00-0		202	101			x
9	Benzo(c)fluorene	205-12-9		216			x	
10	Benz(a)anthracene	56-55-3	4	228	226	113	x	x
11	Cyclopenta(c,d)pyrene	27208-37-3		226	113		x	
12	Triphelylene	217-59-4		228	226	113		
13	Chrysene	218-01-9		228	226	113	x	x
14	5-Methylchrysene	3697-24-3		242	226		x	
15	Benzo(b)fluoranthene	205-99-2	5	252			x	x
16	Benzo(k)fluoranthene	207-08-9		252			x	x
17	Benzo(j)fluoranthene	205-82-3		252			x	
18	Benzo(a)pyrene	50-32-8		252			x	x
19	Indeno(1,2,3-cd)pyrene	193-39-5	6	276	138		x	x
20	Dibenz(a,h)anthracene	53-70-3		278	139		x	x
21	Benxo(g,h,i)perylene	191-24-2		276	138		x	x
22	Dibenzo(a,l)pyrene	191-30-0	7	302			x	
23	Dibenzo(a,e)pyrene	192-65-4		302			x	
24	Dibenzo(a,i)pyrene	189-55-9		302			x	
25	Dibenzo(a,h)pyrene	189-64-0		302			x	

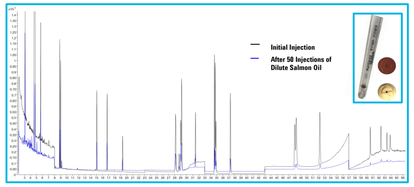

Figure 2. Standard of 50 ng/mL before and after 50 injections of dilute salmon oil

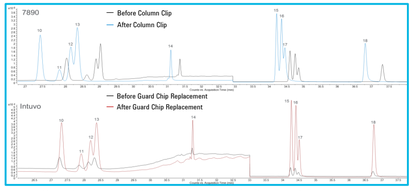

Figure 3. A standard of 50 ng/mL before and after Front End maintenance is performed on a Agilent 7890 GC and an Agilent Intuvo 9000.

Traditional GC to Intuvo 9000 GC

An identical method was applied on an Agilent Intuvo 9000 GC as was used on the Agilent 7890 GC, with the exception of the guard chip, which was not present on the 7890 GC. The Intuvo 9000 utilizes a planer column design and click and run connections.

The same standard containing 25 PAH analytes at 100 ng/mL was analyzed on the Agilent Select PAH GC column designed for a 7890 GC with a traditional 7" cage, and a Select PAH GC column designed for Intuvo 9000 with planar column design. The same temperature program, pressure settings, column phase, and column dimensions were used on the Intuvo 9000 as the 7890 GC. Figure 4 demonstrates that under the identical conditions the two chromatograms are almost indistinguishable with respect to time and peak height.

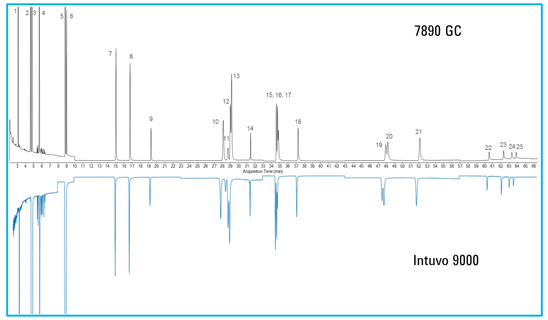

Figure 4. 50 ng/mL standard of 25 PAH compounds analyzed with a Agilent 7890 GC and a Agilent Intuvo 9000 GC.

Easier Front End Maintenance

Ultra Inert Flow Path

The use of the Intuvo 9000 GC with the Ultra Inert Flow Path decreases injection variability due to active compounds, makes it easier to control the system cleanliness, and less downtime due to maintenance.

Decrease Runtime Without Losing Resolution

Figure 5 demonstrates the improved efficiency that is achieved in the analysis of PAH compounds, when decreasing the column length and diameter of the Select PAH GC column. When translating a method to a smaller ID column, it is important to decrease column flow as well as injection volume to avoid overloading the column. The decrease in column flow, from 1.2 mL/min to 0.8 mL/min makes the method more efficient for use with a High Efficiency Source, which has an optimum ionization at a flow of 0.8 mL/min.

By decreasing the column length and the internal diameter, the overall runtime can be decreased by 15% without loss of resolution, allowing for a method that is accurate and efficient.

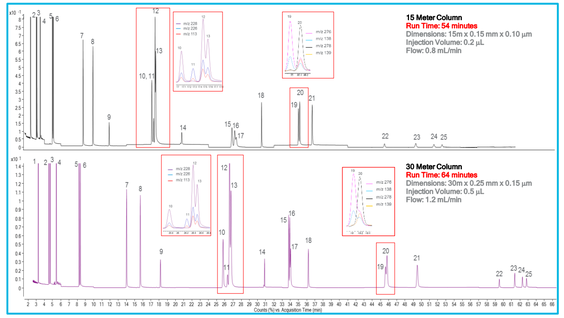

Figure 5. Standard of 25 PAH Compounds analyzed on the Agilent Intuvo 9000 GC using the Select PAH Column with the dimensions of 30m x 0.25 mm x 0.15 µm and 15m x 0.15 mm x 0.10 µm.

Results and Discussion

Enhanced Matrix Removal- Lipid (EMR-Lipid) offers a solution for analyzing samples with high fat content, such as salmon oil. Figure 6 demonstrates the full scan of the same sample of salmon oil prepared by a 1:10 dilution and by using EMR-Lipid, as well as the separation of layers achieved using EMR-Lipid.

The use EMR-Lipid further protects the GC from accumulation in the flow path, and there is a minimal decrease in response for a standard of PAH's, after 50 injections of salmon oil prepared with EMR-Lipid, as demonstrated in Figure 7.

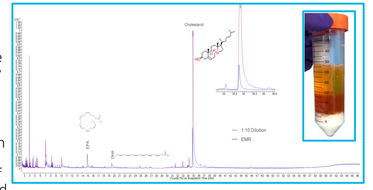

Figure 6. Sample of salmon oil prepared by dilution and EMR and collected in Full Scan mode by GCMS.

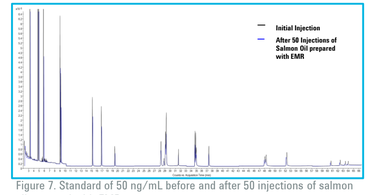

Figure 7. Standard of 50 ng/mL before and after 50 injections of salmon oil prepared with EMR

Conclusions

- Good resolution of 25 PAH compounds is achievable with a Agilent Select PAH GC Column on a traditional 7890 GC and an Intuvo 9000 GC.
- The replacement of a metal microfluidic guard chip on the Intuvo 9000 allows for consistent retention times after front end maintenance.
- Decrease runtime and maintain resolution by decreasing the column length from 30m to 15m and decreasing the carrier gas flow and injection volume
- Use EMR Lipid removal to further clean up sample and increase reproducibility

References

1. K. Lynman. "PAH Analysis with High Efficiency GC Columns: Column Selection and Best Practices" Agilent publication number 5990-8572EN, 2010
2. D. Lucas, L. Zhao. "PAH Analysis in Salmon with Enhanced Matrix Removal" Agilent publication number 5991-6088EN, 2015
3. J. Oostdijk. "Separation of 54 PAHs on an Aglient J&W Select PAH GC Column" Agilent publication number SI-02232, 2010

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