Agilent CrossLab
From Insight to Outcome

Polycyclic Aromatic Hydrocarbons Analysis in Food

Using Triple Quadrupole GC/MS/MS with Helium or Hydrogen Carrier Gases

Consumables Workflow Ordering Guide

Agilent

Polycyclic aromatic hydrocarbons (PAHs) tend to bio-accumulate in fatty foods such as fish, meat, oil and milk, and are extremely toxic even at low concentrations.[1] The US Food and Drug Administration (FDA) requires PAH analysis at low-ppb levels in seafood.[2] The European Union regulates a series of PAHs found in food matrices and provides specific parameters for processed cereal-based food and baby food for infants and young children.[3]

Isolating Analytes from Food Matrix

One of the challenges of analyzing PAHs in fatty food matrices is the extraction of the analytes from the massive amount of lipids found in food matrix. Agilent Captiva Enhanced Matrix Removal (EMR)-Lipid pass through cleanup is the simplest to implement and the most efficient in removing sample matrix. Its selective interaction with the unbranched hydrocarbon chains of lipids makes it ideal for multi-class, multi-residue analysis in fatty-food matrices.[4-6]

GC/MS provides high selectivity and sensitivity for trace levels of PAHs in complex matrices. Although helium is generally considered the best carrier gas for GC/MS analysis, its recurring shortages have increased demand for using hydrogen as the carrier gas. Hydrogen is a reactive gas, and may potentially cause chemical reactions in the inlet, column, and sometimes the MS EI source, which can change analysis results. The Agilent HydroInert source is a newly designed extractor source for GC/MSD that addresses these issues and improves performance with H_2 carrier gas in GC/MS. A method using H_2 carrier gas in the Agilent 8890 GC and 5977 GC/MSD was used to determine PAHs at low concentrations in infant milk, meeting the EU regulation for PAH analysis in food.[4]

Factors to consider when using hydrogen instead of helium as carrier gas

PAHs are relatively durable compounds and therefore can be analyzed with hydrogen carrier gas when using the optimized method and following the recommendations described in these application notes to avoid peak tailing.[7-9]

Table 1. Important factors to consider when using hydrogen carrier gas.

Consideration	Description
Hydrogen Gas	In-house hydrogen, with 99.9999% purity specification and low individual specifications on water and oxygen, is recommended as a carrier gas. It is essential to use a reliable source of clean hydrogen gas. For long-term use, generators with a >99.9999% specification and low individual specifications on water and oxygen are recommended. Moisture filters are recommended for use with hydrogen generators. For short-term use, cylinders with chromatographic or research-grade hydrogen are acceptable.
Pulsed Splitless Injection	Used to maximize transfer of the PAHs, especially the heavy ones, from the GC inlet into the column.
Inlet Liner	The Agilent universal UI mid-frit inlet liner was found to give good peak shape, inertness, and longevity. The frit transfers heat to the PAHs and blocks the line of sight to the inlet base. If the PAHs condense on the inlet base, they are difficult to vaporize and sweep back into the column.
Column Dimensions	Two Agilent J&W DB-EUPAH columns (20 m × 0.18 mm id, 0.14 µm) are recommended to maintain optimal gas flow and inlet pressure in the backflush configuration.
8890 PSD Module and Midcolumn Backflushing	The Agilent 8890 GC pneumatics module is a pneumatic switching device (PSD), optimized for backflushing applications and provides for seamless pulsed injections. The capability to reverse the flow is provided by the Agilent purged Ultimate union (PUU). The PUU is a tee, inserted, in this case, between two identical 20 m columns. During the analysis, a small make-up flow of carrier gas from the 8890 PSD module is required to sweep the connection. During backflushing, the make-up flow from the PSD needs to be raised to a much higher value, to sweep high-boiling contaminants backward out of the first of column and forward from the second.
HydroInert EI Source	The Agilent HydroInert source is a substitute for the extractor source when hydrogen carrier is used. It is constructed with materials that greatly reduce undesirable reactions in the source to maintain spectral fidelity when used with hydrogen. As commonly known, PAHs present unique challenges regarding the MS EI source, even with helium as the carrier gas.[10] With hydrogen carrier gas, the performance of PAHs is improved, especially with the HydroInert source. The 9 mm extractor lens is the default included with the HydroInert source and the best choice for PAH analysis[11,12] as it provides the best calibration linearity, precision of response, and peak shape.
Collision Gas	Only nitrogen should be used as collision gas in GC/TQ when hydrogen is the carrier gas. The collision cell helium inlet fitting must be capped. The optimal nitrogen gas flow is 1.5 mL/min. This flow was also demonstrated to be optimal in previous work on PAHs with hydrogen carrier.[9]
MS/MS	The added selectivity of MRM mode in GC/TQ simplifies the data review of high-matrix samples relative to GC/MS by reducing or eliminating interfering responses from the matrix. Interfering responses often require manual integration of quantifier or qualifier ions.

Separating PAH isomers

One of the challenges of analyzing PAHs is separating PAH isomers by chromatography, as they have the same chemical structure. Mass spectrometers cannot easily distinguish these isomers due to their identical molecular weight. Both the EUPAH4 and wider EUPAH (15+1) include critical pairs that co-elute and are difficult to resolve by GC mass spectrometers. Selecting the right GC column for PAHs depends on the goal of the analysis. Table 2 shows how well the recommended columns can resolve the critical regulated food PAHs and common impurities.

Table 2. Critical regulated PAHs: SCF (PAH15+1), JECFA (PAH13), CONTAM (PAH8).

Analyte list	DB-EUPAH*[13-15]	Select PAH*[16-17]	DB-5ms UI*[16]
Benz[a]anthracene	x	x	x
Cyclopenta[c,d]pyrene	x	x	x
Triphenylene (impurity)	Co-elute	x	Co-elute
Chrysene		x	
Benzo[b]fluoranthene	x	x	Co-elute
Benzo[j]fluoranthene	x	x	
Benzo[k]fluoranthene	x	x	x
Benzo[a]pyrene	x	x	x
Indeno[1,2,3-c,d]pyrene	x	x	x
Dibenzo[a,h]anthracene	x	x	x
Benzo[g,h,i]perylene	x	x	x
Dibenzo[a,e]pyrene	x	x	x
Coronene (impurity)	x	x	x
Dibenzo[a,h]pyrene	x	x	x
Dibenzo[a,i]pyrene	x	x	x
Dibenzo[a,l]pyrene	x	x	x
5-methylchrysene	x	x	x
Benzo(c)fluorene	x	x	x
Total Analysis Time	<28 min[16]	<45 min[17]	<22 min[16]
Maximum Operating Temperature	320 to 340 °C	325 to 350 °C	325 to 350 °C
Business Outcomes	Highest PAH specificity Economical	Highest PAH specificity Productivity	Versatility Productivity
Selection Criteria	– Best choice when resolving triphenylene: chrysene is not critical	– Accurate quantification of all 16 EPA PAHs – Unique selectivity resolves all isomers – Only column separating chrysene from triphenylene, if present	– Economical choice – Excellent for most EPA methods where fewer PAH isomers need to be reported

**x = complete baseline separation*

Molecular weight discrimination

Molecular weight discrimination is another challenge that can occur if:

a. The injection port temperature is set too low (<300 °C) and there is incomplete sample vaporization in the inlet, or

b. The splitless injection hold time is not optimized to effectively transfer all of the sample onto the head of the analytical column, or

c. The wrong inlet liner is chosen. Chromatographically, this will be observed as a lower response of the higher molecular weight PAHs.

Recommendations to avoid molecular weight discrimination and other best practices for optimizing PAH analysis by GC/MS or GC/MS/MS:[10,18]

- Injection volume: 1 to 2 µL
- Inlet, MS source and transfer line temperature: 320 °C. Temperatures below 300 °C will result in PAH tailing. Keep heated zones well insulated and hot to reduce the potential for system cold spots and resultant signal loss.
- Purge time activation: 45 to 90 seconds splitless
- 4 mm splitless liner with mid-frit or glass wool. The frit & wool inside the liner transfers heat to the PAHs and blocks the line of sight to the inlet base. If the PAHs condense on the inlet base, they are difficult to vaporize, and sweep back into the column. Glass-fritted liners are superior alternatives to glass wool as they eliminate the risk of wool breakage or liner movement.
- Pulsed Splitless injection at 20 to 50 psi for 0.9 min to transfer high boiling PAHs onto the column. "Cold trapping" on the liquid phase is often applied for higher molecular, higher boiling analytes such as PAHs for splitless/PTV/MMI type of injections. An initial oven temperature of 75 °C usually provides good quality peak shapes for many sample solvents.
- Use a 0.15/0.18 mm i.d. High Efficiency GC column for faster analysis time with no loss in resolution.
- Minimize inlet (and system) dwell time by operating at higher column flows without compromising MS detector sensitivity. Perform the analysis in constant flow mode.
  0.15 mm: 1.2 mL/min He
  0.18 and 0.25 mm: 1.2 to 1.4 mL/min He
  Note: Although 0.18 mm and 0.25 mm i.d. GC columns can handle higher flow rates, this will lead to decreased MS sensitivity. Exceeding 1.5 mL/min is not recommended for the HES source.
- Use retention gaps and/or backflushing to eliminate sample carry over, reduce maintenance, and cut the analysis cycle times.
- Use Agilent JetClean to substantially reduce the need for manual source cleaning especially with high-matrix samples. Continuous cleaning of the source with hydrogen (0.33 mL/min) has been demonstrated to significantly improve calibration linearity and precision of response over time for PAH analysis.
- A 9 mm extractor lens minimizes the surfaces available for deposition of the PAHs and is the default lens included with the HydroInert source, optimized for use with hydrogen. This is the best choice for PAH analysis, as it provides the best calibration linearity, precision of response, and peak shape.
- Allow PAH standards to come to room temperature before diluting or prepping calibration mixtures since heavier molecular weight PAHs can fall out of solution during refrigerated storage.

References

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16. PAH Analysis with High Efficiency GC Columns: Column Selection and Best Practices, 5990-5872EN
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18. Optimized GC/MS Analysis for PAHs in Challenging Matrices Using the 5977 Series GC/MSD with JetClean and Midcolumn Backflush, 5994-0499EN

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MyList of Sample preparation products

Description	Part No.
Salmon, Beef, Infant formula	
QuEChERS extraction salt packets, original method (10 g samples), no centrifuge tubes, 50/pk	5982-6550
Captiva EMR-Lipid, 3 mL cartridges, with 300 mg sorbent mass, 100/pk	5190-1003
Olive, Grapeseed, Avocado, Almond Oils	
Captiva EMR-Lipid, 6 mL cartridges, with 600 mg sorbent mass, 100/pk	5190-1004
Bond Elut Jr PSA, 500 mg	12162042B
Sample Preparation Supplies	
Ceramic Homogenizers, 15 mL tubes, 100/pk	5982-9312
Centrifuge tube and cap, polypropylene, 15 mL, 25/pk	5610-2039
Centrifuge tube and cap, polypropylene, 50 mL, 25/pk	5610-2049
Positive pressure manifold 48 processor (PPM-48)*	5191-4101
Collection rack, 16 x 100 mm tubes, for PPM-48*	5191-4108
Collection rack, 12 x 32 mm autosampler vials, for PPM-48*	5191-4109
SPE cartridge rack, 3 mL for PPM-48*	5191-4103
SPE cartridge rack, 6 mL, for PPM-48*	5191-4104

One-time purchase.

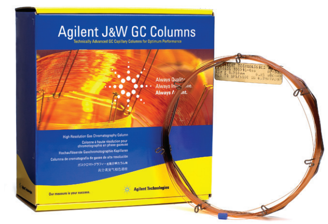
MyList of Standards**

Description	Part No.
Agilent PAH analyzer Calibration Sample Kit	G3440-85009
Deuterated PAH internal standards mix, internal standards	5191-4509

***Please go to www.agilent.com/chem/standards for custom standards.*

MyList of GC columns

Description	Part No.
Agilent J&W DB-EUPAH, 20 m x 0.18 mm x 0.14 µm (Qty: 2, recommended when using hydrogen carrier gas)	121-9627
Agilent J&W Select PAH, 30 m x 0.25 mm, 0.15 µm	CP7462
Agilent J&W Select PAH, 15 m x 0.15 mm, 0.10 µm	CP7461
Agilent J&W DB-5ms UI 20 m x 0.18 mm, 0.18 µm	121-5522UI
Inert fused silica tubing, 5 m, 0.15 mm	160-7625-5



MyList of HydroInert source for transitioning to H_2 carrier gas

Description	Part No.
HydroInert Complete Source Assembly for 5977	G7078-67930
HydroInert Complete Source Assembly for 7000 TQ	G7006-67930
HydroInert GC/MSD Upgrade, contains parts needed to upgrade an existing 5977A/B/C Inert Plus Source	5505-0083
HydroInert GC/TQ Upgrade, contains parts needed to upgrade an existing 7000C/D/E Inert Plus Source	5505-0084
Install Kit for GCs, Stainless Steel, contains 1/8" stainless steel tubing, fittings, Big Universal Trap with stainless steel fittings, and tool kit	19199S

MyList of GC supplies

Description	Part No.
Agilent inlet liner, Ultra Inert, split, low pressure drop, glass wool (recommended for hydrogen gas)	5190-2295
Agilent inlet liner, Ultra Inert, splitless, single taper, glass wool	5190-2293
Advanced Green septum, nonstick, 11 mm, 50/pk	5183-4759
GC inlet seal, gold plated with washer, Ultra Inert, 1/pk	5190-6144
Purged Ultimate Union Assy	G3186-80580
CFT Ferrule Flex Gold flexible metal ferrule, gold plated, 0.4 mm id, for 0.1 to 0.25 mm id fused silica tubing	G2855-28501
ALS syringe, Blue Line, 10 µL, fixed needle, 23-26/42/cone, PTFE-tip plunger	G4513-80203
Ferrule, 0.4 mm id, 15% graphite/85% Vespel, 0.1 to 0.25 mm column, 10/pk	5181-3323
Self Tightening column nut, collared, inlet	G3440-81011
Self Tightening column nut, collared, MSD	G3440-81013

MyList of MS supplies

Description	Part No.
EI filament (for 7000A/B/C/D, 5977B Inert Plus, 5977A extractor, inert or stainless steel and 5975 systems)	G7005-60061
HES Filament for 7010 Triple Quadrupole GC/MS	G7002-60001
Drawout plate, 9 mm, inert	G3440-20022
Drawout plate, 9 mm, extractor source* (for helium carrier gas)	G3870-20449

*G3870-20449 includes a 3 mm drawout plate. For PAH applications replace with the 9 mm drawout plate P/N G3440-20022.

MyList of Gas Clean filters

Description	Part No.
Gas Clean Carrier Gas Kit for 8890 and 8860	CP179880
Gas Clean carrier gas purifier replacement cartridge	CP17973
Gas Clean Filter Kit for Intuvo	CP17995

MyList of Sample containment

Description	Part No.
A-Line screw top vial, 2 mL, amber, write-on spot, 100/pk. Vial size: 12 x 32 mm (12 mm cap)	5190-9590
Cap, screw, blue, PTFE/red silicone septa, 100/pk. Cap size: 12 mm	5182-0717

MyList of Intuvo GC columns

Description	Part No.
Agilent J&W DB-EUPAH Intuvo, 20 m x 0.18 mm, 0.14 µm	121-9627-INT
Agilent DB-UI8270D Intuvo, 30 m x 0.25 mm, 0.25 µm	122-9732-INT
Agilent DB-UI8270D Intuvo, 20 m x 0.18 mm, 0.36 µm	121-9723-INT
Agilent J&W Select PAH Intuvo, 30 m x 0.25 mm, 0.15 µm	CP7462-INT
Agilent J&W Select PAH Intuvo, 15 m x 0.15 mm, 0.10 µm	CP7461-INT
Agilent J&W DB-5ms UI Intuvo, 20 m x 0.18 mm, 0.18 µm	121-5522UI-INT

MyList of Intuvo GC supplies

Description	Part No.
Guard Chip, Intuvo Split/Splitless	G4587-60565
Intuvo inlet chip	G4581-60031
Flow Chip, Intuvo, D2-MS	G4581-60033
Flow Chip, Intuvo, swaged HES MS tail	G4590-60109
Inlet/MSD (Intuvo) Polyimide gasket	5190-9072

Agilent also carries standards for EPA PAH- 500 µg/mL and EU PAH (15+1)- 250 µg/mL and all the GC supplies you would need to analyze PAHs in food matrix reliably and reproducibly, even at trace levels.

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DE.4042824074

Published in the USA, June 8, 2023
5994-2016EN