Agilent CrossLab
From Insight to Outcome

Polycyclic Aromatic Hydrocarbons Analysis in Food

Using triple quadrupole GC/MS/MS: Consumables workflow ordering guide

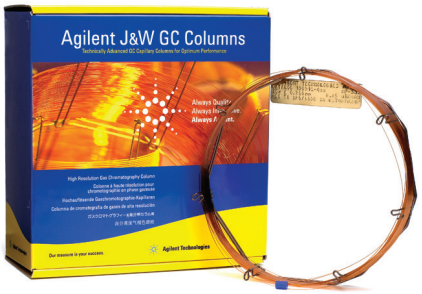
Agilent

Polycyclic aromatic hydrocarbons (PAHs) tend to bio-accumulate in fatty foods such as fish, meat, oil and milk, and are extremely toxic to humans 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 (*3*) a series of PAHs found in food matrices as EU PAH4 (*4*).

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 filtration technology is the simplest to implement and the most efficient in removing sample matrix without analyte loss compared to its predecessors.

Captiva EMR-Lipid filtration technology



Agilent J&W GC columns

References

1. Honda, M., Suzuki, N., Toxicities of Polycyclic Aromatic Hydrocarbons for Aquatic Animals, Int. J. Environ. Res. Public Health **2020**, 17(4), 1363
2. U.S. Food and Drug Administration, 2010, accessed July 2020, Protocol for Interpretation and Use of Sensory Testing and Analytical Chemistry Results for Re-opening Oil-Impacted Areas closed to Seafood Harvesting due to the Deepwater Horizon Oil Spill.
3. Commission Regulation (EU) 836/2011, Official Journal of the European Union, **2011**, 215, 9
4. Commission Regulation (EU) No 835/2011, Official Journal of the European Union, **2011**, 215, 4

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 coelute and are difficult to resolve by GC mass spectrometers. Selecting the right GC column for PAHs depends on the goal of the analysis. Table 1 shows how well the recommended columns can resolve the critical regulated food PAHs and common impurities.

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

Analyte list	DB-EUPAH*	Select PAH*	DB-5ms UI*
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	x
Benzo[j]fluoranthene	x	x	co-elute
Benzo[k]fluoranthene	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[1]**	**<45 min[2]**	**<22 min[1]**
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*
1. Refer to application note number 5, on page 6.
2. Refer to application note number 8, on page 6.

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.

Recommended injection parameter ranges to avoid molecular weight discrimination:

- injection volume: 1 to 2 µL
- inlet temperature: 300 to 320 °C
- MS source and transfer line temperature: 320 °C
- purge time activation: 45 to 90 seconds splitless
- 4 mm splitless liner with glass wool or fritted liners
- Pulsed Splitless 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
- Minimize inlet (and system) dwell time by operating at higher column flows: 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.

Best practices for optimizing triple quadrupole GC/MS systems (GC/TQ) for PAH analysis

- Use retention gaps and/or backflushing to eliminate sample carry over, reduce maintenance and cut the analysis cycle times
- Perform the analysis in constant flow mode
- Keep heated zones well insulated and hot to reduce the potential for system cold spots and resultant signal loss
- Keep MS transfer line and ion source at temperatures above 300 °C. Too low temperature settings will result in PAH tailing Use a 0.15/0.18 mm i.d. high efficiency GC columns for faster analysis time with no loss in resolution
- 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
- 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
- Use a 9 mm extractor lens to minimize the surfaces available for deposition of the PAHs.

Application notes

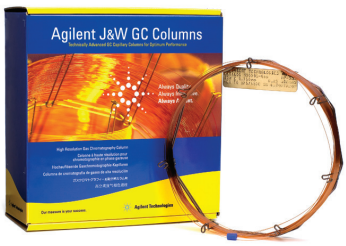
For sample preparation conditions and GC method optimization details, see the following Agilent application notes:

1. Determination of 19 polycyclic aromatic hydrocarbon compounds in salmon & beef using Captiva EMR-Lipid cleanup by GC/MS/MS
2. Determination of 14 Polycyclic Aromatic Hydrocarbon Compounds in Edible Oil
3. PAHs in Chocolate and Peanuts with Agilent J&W Select PAH and longer GC columns
4. Polycyclic Aromatic Hydrocarbon (PAH) Analysis in Fish by GC/MS using Agilent Bond Elut QuEChERS dSPE Sample Preparation and a High Efficiency DB-5ms Ultra Inert GC column
5. PAH Analysis with High Efficiency GC columns: Column selection and Best practices
6. Increased Reproducibility in the Analysis of EU and EPA PAHs with the Agilent J&W Select PAH GC column and Agilent Intuvo 9000 GC system
7. GC/MS Analysis of European Union (EU) priority Polycyclic Aromatic Hydrocarbons (PAHs) using and Agilent DB-EUPAH GC column with a column performance comparison
8. Separation of 54 PAHs on an Agilent J&W Select PAH GC columns
9. Different Stationary Phases for PAH Analysis (Food Quality & Safety magazine)
10. Analysis of European Union Polyaromatic Hydrocarbons (EUPAH) with Agilent 8890GC
11. Optimized GC/MS Analysis for PAHs in Challenging Matrices using the 5977 Series GC/MSD with JetClean and midcolumn backflush
12. Optimized GC/MS/MS Analysis for PAHs in Challenging Matrices using the Agilent 8890/7000D triple quadrupole GC/MS with Jet Clean and midcolumn backflush

Recommended products for PAH analysis of food samples

The table, following, lists products needed for PAH analysis from food matrices. Click here to add all the items in this list to your My Favorites list on the Agilent online store. * Alternatively, click the 'MyLists' link in each table heading to add all those items your My Favorites list. You can then enter the quantities for the products you need. Your list will remain under My Favorites for you to use with future orders.

Item	Part Number
View MyList of sample preparation supplies	
Captiva EMR-Lipid cartridge, 3 mL, 300 mg	5190-1003
Ceramic homogenizers	5982-9312
Agilent positive pressure manifold processor (PPM-48)	5191-4101
Captiva EMR-Lipid cartridge, 6 mL, 600 mg	5190-1004
Bond Elut Jr PSA, 500 mg	12162042B
View MyList of standards	
EU PAH(15+1) standard kit; 250 µg/mL	5190-0487
EPA PAH standard (calibration standard); 500 µg/mL	8500-6035
Please go to www.agilent.com/chem/standards for additional volume and concentration standard options	
View MyList of Inlet Supplies	
Inlet septa, Advanced green, nonstick, 11 mm, 50/pk	5183-4759
Inlet septa, Advanced green, nonstick, 11 mm, 100/pk	5183-4759-100
Ultra Inert Splitless, single taper, glass wool	5190-2293
Ultra Inert splitless single-taper liner with glass frit	5190-5112
Ultra Inert Gold seal, with washer, 1/pk	5190-6144
Ultra Inert Gold seal, with washer, 10/pk	5190-6145
Self-Tightening column nut, collared, inlet	G3440-81011
Self-Tightening column nut, collared, MSD	G3440-81013
Replacement collar for self tightening nut	G3440-81012
15% Graphite / 85% Vespel Ferrules, 0.4 mm i.d., 10/pk	5181-3323
5 µL ALS syringe, fixed needle, 23-26s/42/cone	5181-1273
5 µL ALS syringe, fixed needle, 23-26s/42/cone 6/pk	5181-8810
10 µL ALS syringe, fixed needle, 23-26s/42/cone	5181-1267
10 µL ALS syringe, fixed needle, 23-26s/42/cone 6/pk	5181-3360
20x magnifier loop	430-1020
View MyList of GC Columns- 7890, 8890 and 8860	
Agilent J&W DB-EUPAH, 20 m x 0.18 mm, 0.14 µm	121-9627
Agilent J&W DB-5ms 20 m x 0.18 mm, 0.18 µm	121-5522UI
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
Inert fused silica tubing, 5 m, 0.15 mm	160-7625-5



If this is your first time using the Agilent online store, you will need to enter your email address for account verification. If you don't have a registered Agilent account, you will need to register for one. The "My List" feature is valid only in regions that are e-commerce enabled. All items can also be ordered through your regular sales and distributor channels. Not available in all countries. Please contact your local sales representative for availability.

View MyList of Intuvo GC columns	
Agilent J&W DB-EUPAH, Intuvo GC column 20 m x 0.18 mm, 0.14 µm	121-9627-INT
Agilent J&W DB-5ms 20 m x 0.18 mm, 0.18 µm	121-5522UI-INT
Agilent J&W Select PAH, Intuvo GC column, 30 m x 0.25 mm, 0.15 µm	CP7462-INT
Agilent J&W Select PAH, 15 m x 0.15 mm, 0.10 µm	CP7461-INT
View MyList of Intuvo supplies	
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
View MyList of MS supplies	
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 source	G3440-20022
Drawout plate, 9 mm, extractor source	G3870-20449
View MyList of gas filters	
Gas Clean Carrier Gas Kit for 7890	CP17988
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
View MyList of vials and caps	
2 mL screw top amber, write-on spot, deactivated, certified, 100 pc	5183-2072
Screw Caps, blue, certified, PTFE/silicone/PTFE septa	5182-0723
100 µL vial insert, glass with polymer feet	5181-8872
2 mL, screw top, amber, write-on certified, 100/pk	5182-0716
9 mm blue screw caps, PTFE/RS, 500/pk	5185-5820

To learn more visit: www.agilent.com/chem/standards

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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