PAHs in Chocolate and Peanuts with Agilent J&W Select PAH and Longer GC Columns

Application Note

Food Testing & Agriculture

Author

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Abstract

Separation of isomeric polycyclic aromatic hydrocarbons (PAHs) at ppb level using GC/MS in SIM mode permits the analysis of cocoa and peanut extracts using Agilent Bond Elut SPE cleanup with co-extracted triglycerides present in the injected sample matrix. PAHs comprise a large group of chemical compounds known to be cancer-causing agents. They can occur in food either by uptake from the environment or are generated during food processing. Numerous PAHs have been demonstrated to be mutagens as well. By using a relatively short 15 m Agilent Select J&W PAH GC column, a run time of less than 1 hour was achieved with a reasonably high temperature bakeout at the end of the run. A sample prep procedure validated for HPLC with fluorescence was adapted for use with GC/FID and GC/MS analysis. Sample extracts were screened in scan mode to demonstrate the presence of late eluting potential interferences. Then fortified chocolate samples were compared to native chocolate to demonstrate the presence or absence of reportable trace level PAHs in SIM mode. The Select PAH column in a 15 m geometry offered good separation of even difficult peak pairs, while permitting the shortest bakeout between injections, making this column more rugged in long-term use. Options were discussed about the possibility of shortening run times using backflush with capillary flow technology (CFT).

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Introduction

Recent dietary exposure to PAHs has brought with it greater focus on the food items requiring attention. A majority of these contain high dietary fats and triglycerides. While it is established that grilling over an open flame will introduce PAHs to many foods, roasting processes such as those used for cocoa beans and legumes, such as peanuts, offer another means for PAHs to be introduced. Separation of trace levels from these matrixes can be challenging and time consuming [2, 3]. This application note offers a reasonable path to extract and analyze samples for PAHs. To preserve GC column lifetime, it is recommended that an Agilent Ultra Inert liner with wool be used to collect co-extracted nonvolatiles at the injection port liner. Total run times on the gas chromatography assay were approximately 60 minutes. The complete elution of all PAHs was 30 minutes and additional column bakeout of co-extracted cocoa butter tiglycerides required another 30 minutes. The J&W Select PAH GC column, along with an optimized temperature program [1], permitted separation of the isomeric components and provided the shortest overall assay time.

Materials and Methods

An Agilent 7890A GC was coupled to an Agilent 5975C Series GC/MSD with the inert EI 350 noncoated source and tandem axis detector. A similar setup, using an FID detector, was used to evaluate longer columns to establish preliminary adequate bakeout times.

GC conditions

Column:	Agilent J&W Select PAH, 15 m × 0.15 mm, 0.10 µm (p/n CP7461)
Sample prep:	Solid phase extraction with Agilent Bond Elut SI, 1 g/6 mL (p/n 12256008)
Sample:	1 g finely chopped milk chocolate bars or shelled oil-roasted peanut
Carrier:	MSD UHP Helium, FID Hydrogen, both at 1.2 mL/min constant flow
Oven:	70 °C (hold 0.4 min) to 180 °C at 70 °C/min, then to 230 °C at 7 °C/min (hold 7 min), then to 280 °C at 50 °C/min (hold 7 min), then to 350 °C at 30 °C/min (hold 24 min)
Injection:	Splitless, 100 mL/min split flow on at 0.5 min, gas saver on at 2 min
Inlet temperature:	300 °C
MSD transfer aux temperature:	350 °C
Detector:	FID at 350 °C
Sampler:	Agilent 7693A Automatic Liquid Sampler 1 µL injection volume

MS conditions

Solvent delay: 1.4 min

MS temperature: 300 °C (source), 150 °C (quad)

SIM mode:

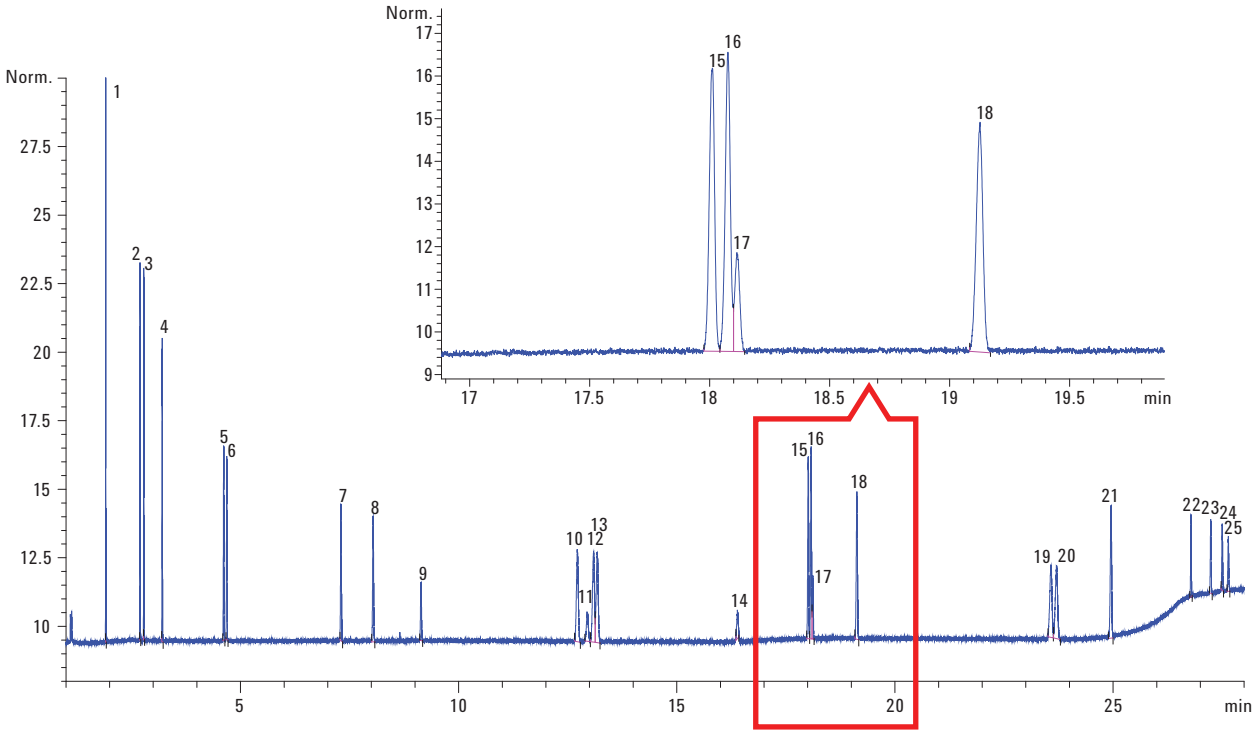
Group	Start time	Ions	Dwell (ms)
1	1.40	128, 152, 153, 165	50
2	4.70	178	200
3	7.40	202, 216	100
4	12.50	226, 228, 242	50
5	18.30	252	200
6	23.00	276, 278	100
7	27.00	302	200

Flow path supplies

Ferrule:	Long, 0.3 mm (p/n 5062-3507)
Vials:	Amber, screw cap (p/n 5182-0716)
Caps:	Blue, screw cap (p/n 5282-0723)
Vial inserts:	250 µL glass with polymer feet (p/n 5181-1270)
Syringe:	5 µL (p/n 5181-1273)
Septum:	Red Bleed Temp Optimized BTO (p/n 5183-4757)
Inlet liner:	Universal Ultra Inert with wool (p/n 5190-2295)

Standards

A 25-component mix of the 15+1 European Food Safety Authority (EFSA) targets with an additional nine components from United States Food & Drug Administration (USFDA) listed targets was prepared by combining two reference mixes (p/n 5190-0487 and 8500-6035). Triphenylene was purchased from Sigma-Aldrich Corp. (p/n 442830, 5 mg). Figure 1 demonstrates that separation was acceptable for all components in the standard mix with difficult isomeric peaks Benzo[b,k,and j] fluoranthene (15 thru 17) resolved.



Peak ID

1) Naphthalene	6) Anthracene	11) Chrysene	16) Benzo[k]fluoranthene	21) Benzo[ghi]perylene
2) Acenaphthylene	7) Fluoranthene	12) Cyclopenta[cd]pyrene	17) Benzo[j]fluoranthene	22) Dibenzo[a,l]pyrene
3) Acenaphthene	8) Pyrene	13) Triphenylene	18) Benzo[a]pyrene	23) Dibenzo[a,e]pyrene
4) Fluorene	9) Benzo[c]fluorene	14) Chrysene, 5-methyl-	19) Dibenz[a,h]anthracene	24) Dibenzo[a,i]pyrene
5) Phenanthrene	10) Benz[a]anthracene	15) Benzo[b]fluoranthene	20) Indeno[1,2,3-cd]pyrene	25) Dibenzo[a,h]pyrene

Figure 1. FID trace of 25-component PAH evaluation mix at 10 µg/mL (10 ng each) with optimized temperature program on an Agilent J&W Select PAH GC column.

Sample preparation [2,3]

1. Slurry 1 g chocolate in 10 mL MeOH and grind to fine solids with PTFE-coated steel spatula to defat/extract cocoa butter and PAHs.
2. Allow the cocoa solids to settle (1 hour), remove MeOH, and evaporate to dryness under lab N_2.
3. Wash the solids with 2 mL fresh MeOH.
4. Resuspend the oily residue in 10 mL deionized water.
5. Liquid-liquid extract the oily residue deionized water with 5 mL (x2) of *n*-pentane, combine the 5 mL portions and dry under lab N_2.
6. Redissolve the *n*-pentane residue in 2 mL *n*-pentane.
7. SPE - precondition 1 g/6 mL Bond Elut SI SPE tubes with 5 mL MeOH, then with 5 mL THF, and, finally, with 5 mL *n*-pentane. Apply the 2 mL pentane extract, wash tube with 3 mL clean *n*-pentane, elute the 5 mL total.
8. Elute the PAHs with 10 mL (5 mL × 2) 10% MeOH:90% THF (uninhibited).
9. Evaporate to near dryness and reconstitute to the final volume of 1 mL of MeOH for injection.

Results and Discussion

Numerous laboratories have measured PAHs in a variety of samples. The main contributors to dietary PAH exposure for adults are fats, bread, and dried bread products, followed by crustaceans and mollusks. A screening method has been developed at the USFDA for shellfish using QuEChERS extraction with HPLC and fluorescent detection [5]. Early adopters have had good success with this technique. Improvement in selectivity for this assay can be achieved using LC/MS with APPI and a toluene doping gas[6]. French Institut National Recherche Agronomique (INRA) labs have developed a good extraction using SPE and coupled that to HPLC with fluorescence as well [2]. The GC/MS approach allows laboratories engaged in this area to gain further confidence and could be useful to develop better sample cleanup strategies as this assay evolves. At the source of importation for vast amounts of cocoa beans from India, authors at Council of Scientific & Industrial Research (CSIR) describe using SPE to recover PAHs from silica gel with good success [3]. None of these sample extraction strategies takes into account complete removal of the triglycerides that are potential interferences in GC/MS, as seen in Figures 2 and 3. It is possible to remove interferences using an automated GPC cleanup procedure [4]. It would also be possible to incorporate a capillary flow technology (CFT) backflush to shorten run times even further and perhaps reduce mass spectrometer source contamination during bakeout. The European Food Safety Authority (EFSA) requires less than 30 µg/kg in most foodstuffs entered into commerce as calculated by PAH [4]. This is the sum total of four PAHs; BaP (benzo[a]pyrene), BaA (benz[a]anthracene), CHR (chrysene), and BpFl (benzo[b]fluoranthene). Figure 4 shows that the chocolate samples analyzed do not have measurable traces of isomeric PAHs benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, and benzo[a]pyrene.

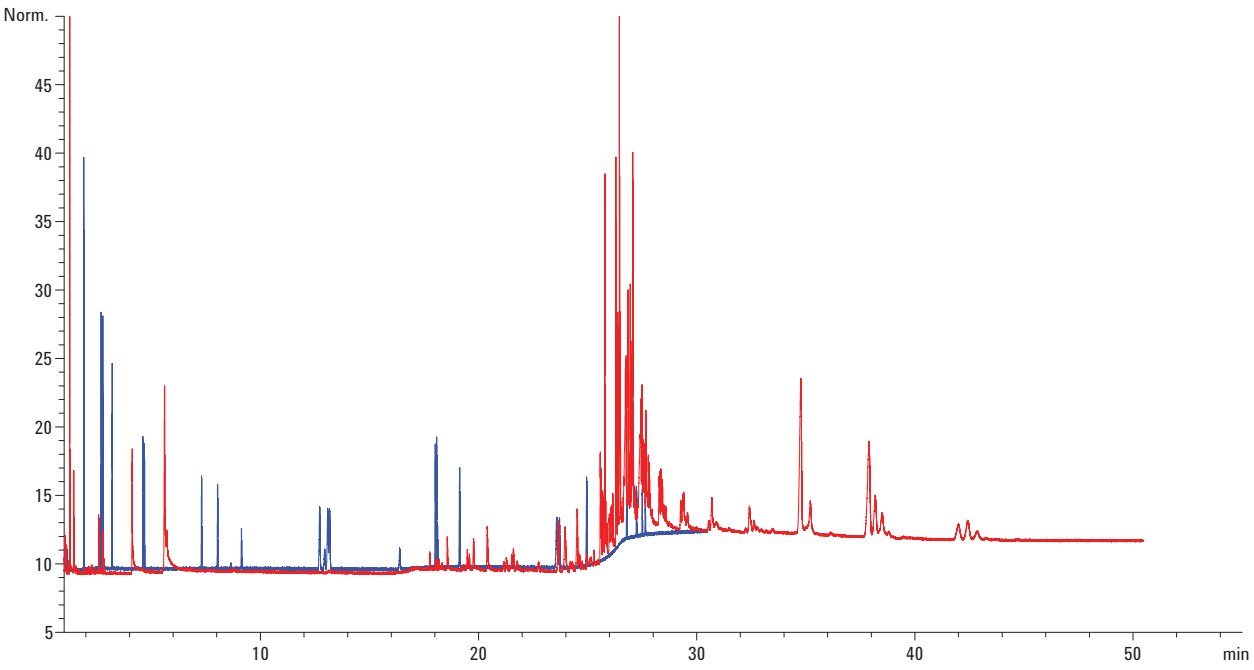


Figure 2. Overlay of milk chocolate extract (red trace) matrix with PAH standards (blue trace) showing interferences and need for bakeout at end of run. The column was the Agilent J&W Select PAH 15 m × 0.15 mm, 0.10 µm film.

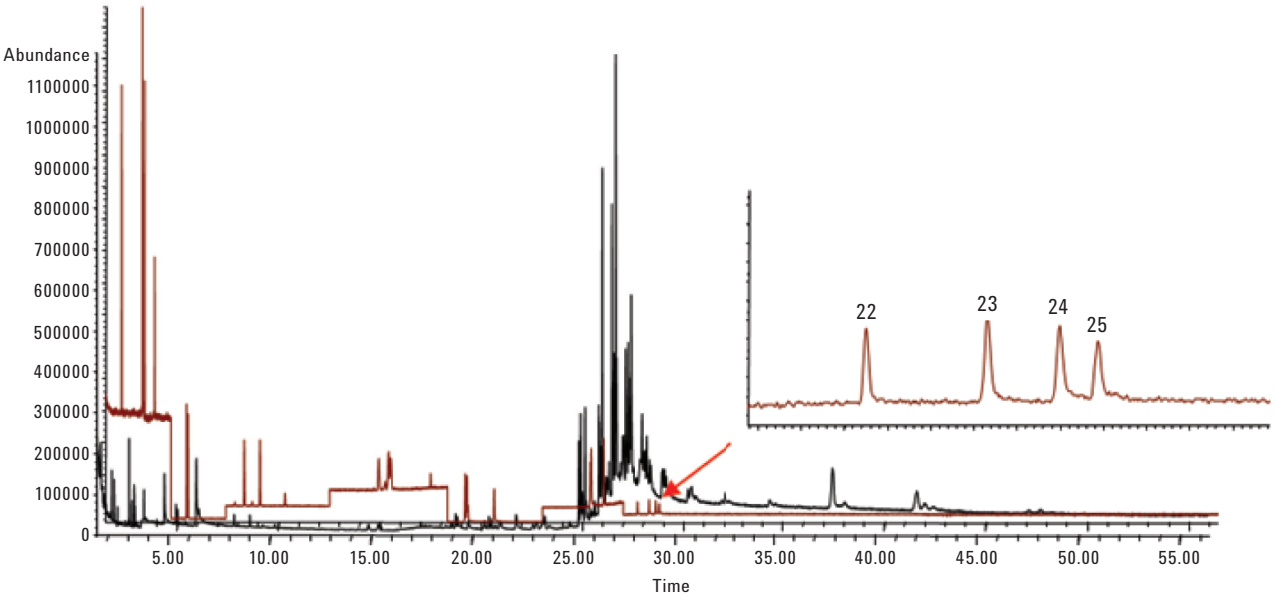


Figure 3. Overlay of milk chocolate extract (black trace) matrix with PAH standards (red trace) showing good response at 10 ppb with last components having no interfering ions. The column was the Agilent J&W Select PAH 15 m × 0.15 mm, 0.10 µm film.

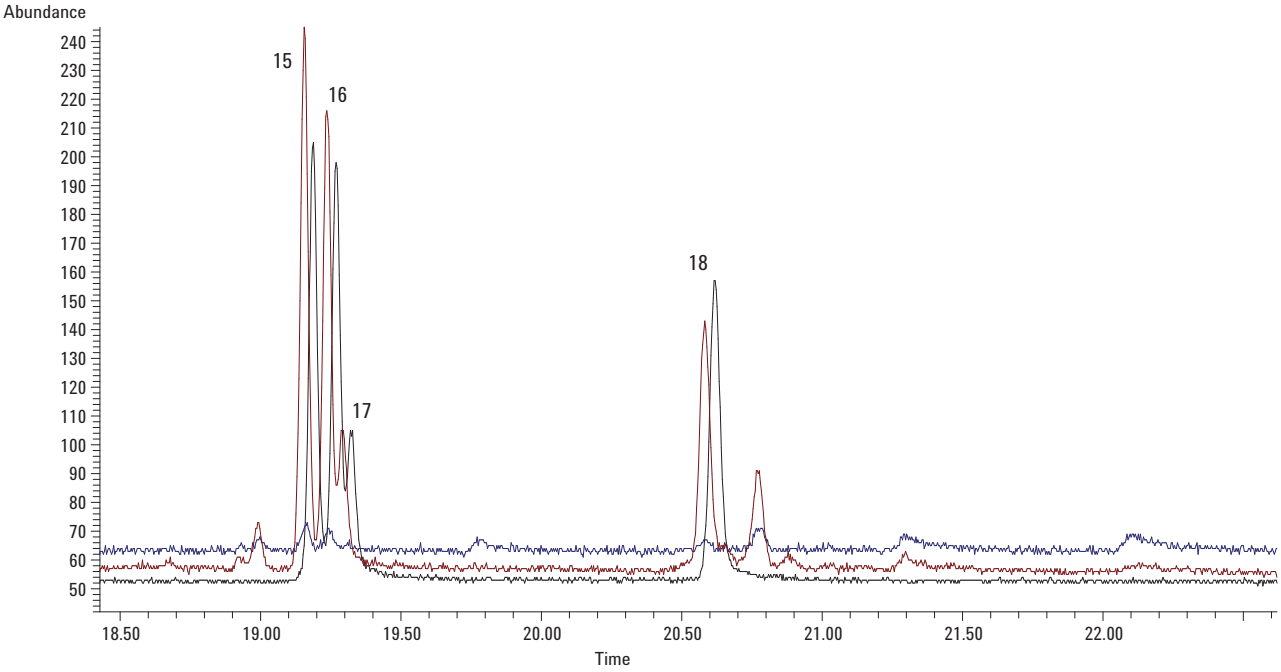


Figure 4. Overlay of 10 ppb standard (black trace), 10 ppb fortified chocolate sample (red trace), and native chocolate sample (blue trace) demonstrating absence of critical isomeric PAHs benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[j]fluoranthene, and benzo[a]pyrene, using an Agilent J&W Select PAH GC column.

Conclusions

The Agilent J&W Select PAH GC column with an optimized temperature program provides satisfactory separation of isomeric PAHs to meet regulatory requirements. By virtue of being only 15 meters in length, it also allows for a reasonable bake out time between runs for real-world sample extracts, including high triglyceride milk chocolate and oil-roasted peanuts. A possible alternative strategy could be to install a capillary flow technology back flush system and execute a short backflush during bake out starting at approximately 30 minutes. This could shorten the bake out time by approximately 20 minutes. Longer columns require lengthy bake out and could potentially degrade from extended time spent at high temperatures, introducing loss of critical resolution of isomeric pairs. Upon completion of a typical sample group (10 samples) the mass spectrometer tune was verified, indicating the instrument was able to operate satisfactorily. Lastly, the instrument was vented and the source was visually inspected. The source had become dirty and was subjected to in-house cleaning. Source maintenance will be required less often if backflush using CFT is used.

References

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