Analysis of PAHs in Infant Formula and Blueberries Using QuEChERS and GCxGC-TOFMS

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Key Words: GCxGC-TOFMS, PAH, Food Analysis, QuEChERS

1. Introduction

PAHs are widespread carcinogenic pollutants that are naturally found in crude oil and coal deposits but are primarily formed through incomplete combustion processes. Due to the health risks posed, identifying their presence in foodstuffs is of great importance. Up to 70% of a non-smoker's non-occupational exposure to PAHs have been linked to food sources.[1] The QuEChERS[2] method of sample preparation is used in this study because it is a fast and inexpensive food matrix extraction method. The LECO Pegasus® 4D (GCxGC-TOFMS) instrument was used to isolate the PAHs from matrix interferents and produce EI mass spectra for identification.

2. Experimental Conditions

Sample Preparation

The PAH standards analyzed were from a 2000 µg/mL phenanthrene-d10 in dichloromethane internal standard (Restek #31045), 500 µg/mL EPA Method 8310 PAH mixture in acetonitrile (#31841), and 1000 µg/mL decafluorobiphenyl in acetonitrile (#31842). For each spiked 10 g sample of liquid infant formula or blended blueberries, 100 µL of each standard solution was injected.

QuEChers Methodology

- Weigh 10 g of sample into clean 50 mL centrifuge tube
- Add 10 mL acetonitrile
- Shake vigorously for 1 minute
- Add Qsep 150 extraction salts
- Shake vigorously for 1 minute
- Centrifuge at 3000 g for 5 minutes
- If using dSPE kit, continue. Otherwise, transfer supernatant to autosampler vial and analyze.
- Transfer 1 mL of supernatant to dSPE Qsep250 tube
- Shake vigorously for 30 seconds
- Centrifuge at 3000 g for 5 minutes
- Immediately adjust pH using 10 µL of a 5% formic acid in acetonitrile solution
- Transfer supernatant to autosampler vial and analyze

Analysis Methodology

A split/splitless inlet was used with 1.40 mL/min helium carrier gas flow. The 1 µL injected sample was analyzed in the splitless mode. The column set used was a 30 m x 0.25 mm x 0.25 µm Rxi-1ms primary column and a 1.0 m x 0.10 mm x 0.10 µm RTX-17 secondary column. The main oven temperature program was a 30 s hold at 50°C followed by a 10°C/min ramp to 300°C followed by a 10 min hold. The secondary oven mirrored the ramp at +10°C. The modulation period was 5 s with 0.80 s hot pulse per stage.

The MS collected masses 50-500 m/z at a rate of 100 spectra/s. Electron energy for the EI was -70 V and the detector voltage was 1600 V. The ion source temperature was 200°C. An acquisition delay of 500 s was used.

3. Results

Table 1 provides the primary retention time (tr') and secondary retention time (tr") results in both dimensions along with peak number, unique mass, and detection limit based on extrapolation to signal-to-noise (S/N) of 5. The three critical pair candidates under these separation conditions are phenanthrene/anthracene (9/10), benzo(b)fluoranthene/benzo(k)fluoranthene (15/16), and indeno(1,2,3-cd)pyrene/dibenzo(a,h)anthracene (18,19). Compounds that elute early tended to have lower detection limits, as there was less primary column band broadening and thus more material focused per modulation for reinjection into the secondary column.

Table 1: PAH target compounds listed in general elution order with peak #, retention times, unique masses, and detection limits.

Analyte	Peak #	Unique Mass	Avg tr'	Avg tr''	pg
decafluorobiphenyl	1	334	625	0.75	2.3
naphthalene	2	128	685	1.06	0.7
2-methylnaphthalene	3	142	778	1.06	1.5
1-methylnaphthalene	4	142	790	1.09	1.1
acenaphthylene	5	152	895	1.20	1.2
acenaphthene	6	153	920	1.19	1.7
fluorene	7	166	995	1.19	2.3
phenanthrene-d10	8	188	1120	1.33	2.5
phenanthrene	9	178	1120	1.34	2.0
anthracene	10	178	1130	1.31	2.1
fluoranthene	11	202	1285	1.43	1.8
pyrene	12	202	1315	1.50	1.8
benzo(a)anthracene	13	228	1480	1.59	6.4
chrysene	14	228	1485	1.62	23.5
benzo(b)fluoranthene	15	252	1625	2.16	20.4
benzo(k)fluoranthene	16	252	1630	2.16	4.7
benzo(a)pyrene	17	252	1670	2.57	25.1
indeno(1,2,3-cd)pyrene	18	276	1875	4.08	48.4
dibenzo(a,h)anthracene	19	276	1880	4.05	93.6
benzo(ghi)perylene	20	276	1928	4.72	150.0

Figure 1 displays the GCxGC contour plot of the PAH-spiked infant formula sample without the use of dSPE cleanup. The twenty components that were spiked into the infant formula are labeled with numbers on the plot which correspond to peak # in Table 1. The second-column separation easily differentiates the PAHs from the infant formula matrix. The modulation period of 5 s is required to avoid having peaks 18, 19, and 20 wrap around. However, without those compounds present, the analysis could be performed using a 3 s modulation period instead.

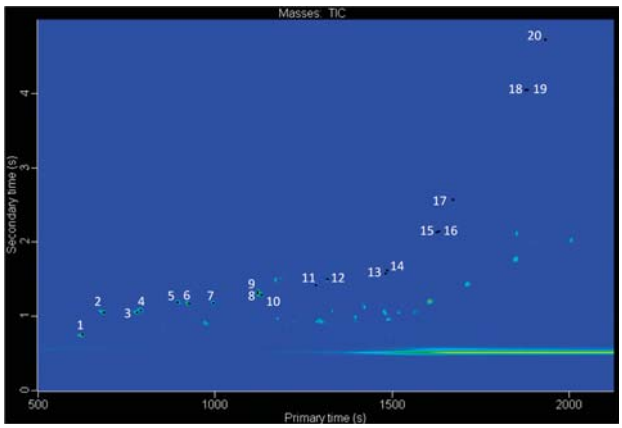

Figure 1. GCxGC contour plot of PAH-spiked, QuEChERS-extracted infant formula sample. The peak # labels on the plot correlate to the Peak # entries in Table 1.

Figure 2 displays the GCxGC contour plot of the infant formula matrix without PAH spiking. The prominent matrix peaks are primarily organic acids such as dodecanoic acid, but also include nonyl acetate, vitamin E acetate, and cholesterol. With some exceptions near the end of the chromatogram, there are no intense peaks in the TIC that elute beyond the 2 s tr" in the second dimension.

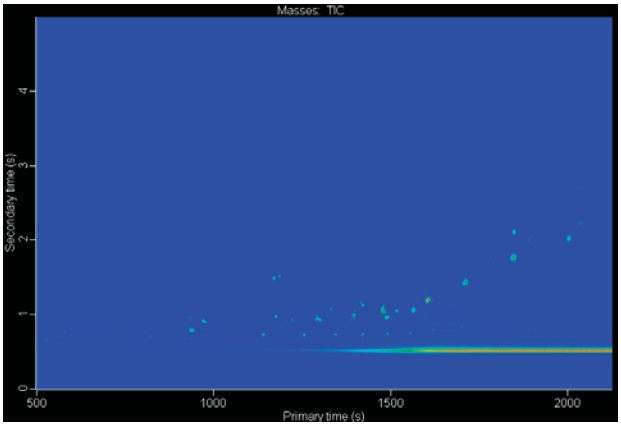

Figure 2. GCxGC contour plot of non-spiked QuEChERS-extracted infant formula sample.

Figure 3 displays the GCxGC contour plot of the PAH-spiked blueberry sample, also without the use of dSPE cleanup. The twenty components that were spiked into the blended blueberries are labeled with numbers on the plot which correspond to peak # in Table 1. Similar to the infant formula matrix, the second-column separation prevents the PAH contaminants from coeluting from blueberry matrix peaks.

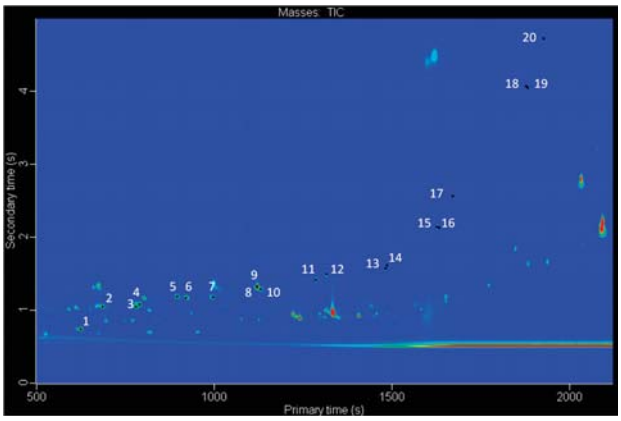

Figure 3. GCxGC contour plot of PAH-spiked, QuEChERS-extracted blueberry sample. The peak # labels on the plot correlate to the Peak # entries in Table 1.

Figure 4 displays the GCxGC contour plot of the blueberry matrix without PAH spiking. The prominent matrix peaks include 2-methoxyphenol, phenylethyl alcohol, 2-undecanone, ethyl dodecanoate, tetradecanal, n-hexadecanoic acid (palmitic acid), (E)-9-Octadecenoic acid ethyl ester, (Z)-9-octadecenamide, isopropyl linoleate, pentadecanal, 1-docosene, octadecanal, and sitosterol.

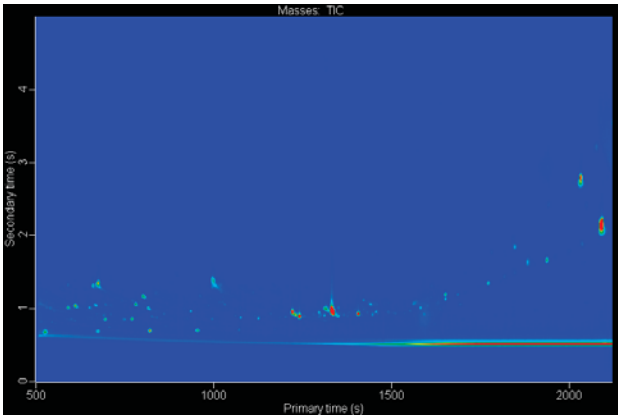

Figure 4. GCxGC contour plot of non-spiked QuEChERS-extracted blueberry sample.

Figure 5 displays PAH standard calibration curves for pyrene in the 10-50 pg range and 0.5-5 ng of on-column material. The linearity shown by the trendlines are sufficient for the regions shown. The calibration curves were collected with different MS optimizations, hence the separate curves.

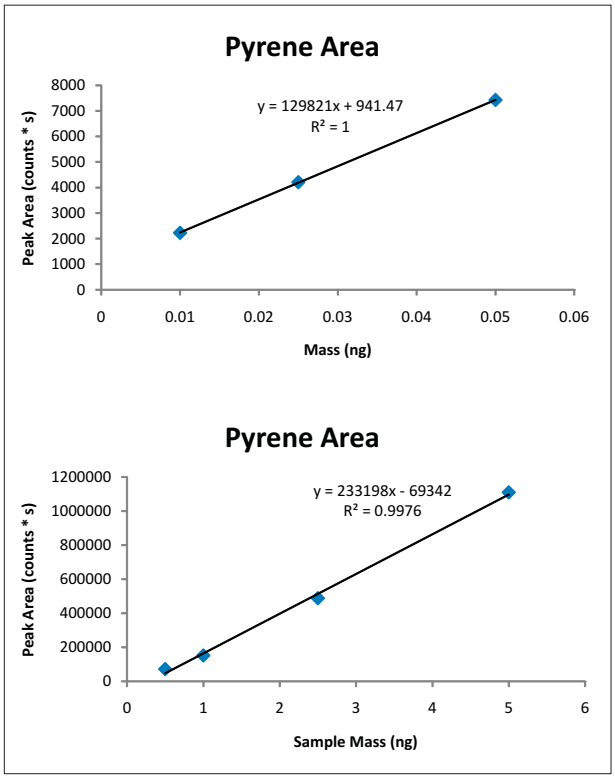

Figure 5. Calibration curves for the PAH standard pyrene. The top plot covers the range of 10-50 pg and bottom plot covers the range of 0.5-5 ng. The curves are separated into two because different MS optimizations were used during data collection.

4. Conclusions

The combination of QuEChERS extraction and GCxGC-TOFMS is an excellent method for detecting and identifying PAH contaminants in foodstuff matrices such as liquid infant formula and blended blueberries. This is a fast process, as the total procedure (extraction and analysis) was completed within the space of an hour. Rapid analysis techniques that can isolate PAHs from complicated food matrices such as these can be a valuable tool for time-sensitive situations such as possible food recalls.

5. References

[1]Skupinska K.; Misiewicz, I.; Kasprzycka-Guttman T. 2004. Polycyclic aromatic hydrocarbons: physiochemical properties, environmental appearance and impact on living organisms. Acta Pol Pharm 61 (3):233-40.

[2]http://www.restek.com/pdfs/805-01-002.pdf

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