Significant Robustness Improvements of PAHs Analysis in Palm Oil Using the JetClean Self-Cleaning Ion Source in a GC/MS/MS System

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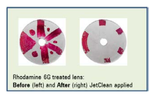
Introduction

Regulated limits for PAHs (polyaromatic hydrocarbons) in food have been steadily lowered as the result of increasing awareness about their potential carcinogenic impact and their presence in our food supply. The allowed maximum contaminant levels in critical matrices are set as low as 1 µg/kg, as indicated by European Regulation 1881/2006, shown below.

Foodstuffs	Maximum levels (µg/kg)	
Benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene	Benzo(a)pyrene	Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene (*5)
Oils and fats (excluding cocoa butter and coconut oil) intended for direct human consumption or use as an ingredient in food	2,0	10,0
Dietary foods for special medical purposes (*5,*6) intended specifically for infants	1,0	1,0

To achieve these detection levels consistently in complex food matrices, EI (electron ionization) sources typically require frequent cleaning, leading to lost laboratory productivity.

The JetClean Self-cleaning ion source with automatically controlled hydrogen addition eliminates the need for frequent manual cleaning and assures consistent results over multiple weeks, even after many months of operation.



Rhodamine 6G treated lens:
Before (left) and **After** (right) JetClean applied

Rhodamine G6

To demonstrate the effectiveness of JetClean, the extractor lens was heavily marked with a Sharpie pen, creating a serious Rhodamine 6 "contamination" on the lens, imitating the accumulation of deposits one would see during normal operation in the analysis of samples with heavy matrices. After an automatically initiated cleaning cycle utilizing prudently controlled hydrogen flows the deposits from the lens were removed without human interaction.

Experimental

Sample preparation: Palm oil, a common food component and challenging matrix, was extracted by toluene without any purification. The extract was spiked at 5 ng/ml of each of the regulated 4 PAHs, resulting in an equivalent to 1 µg/kg of Benzo(a)pyrene (BaP), Benzo(a)anthracene (BaA) , Chrysene, and Benzo(b)fluoranthene (BbF) in palm oil. ^{13}C labels of the analytes were added as the internal standard.

Instrumentation: The analysis was performed using Agilent's 7000C GC/MS/MS-based PAH Analyzer, with a pulsed splitless injection at 320°C, a DB-EUPAH column (30m x 0.25mm, 0.25µm) and with post-column backflushing. The column oven was ramped from 80°C to 335°C during a 25 minute run.

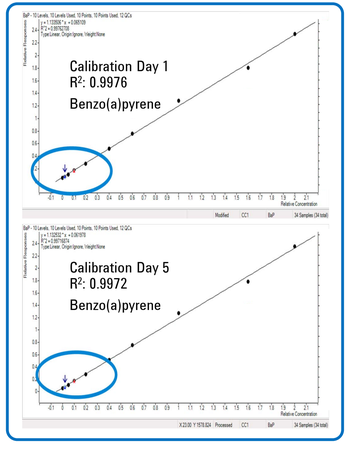
The source and quadrupole temperatures were 320°C and 180°C respectively and a 9mm extractor lens was used. To achieve the most robust operation, the JetClean applied continuous H_2 flow. One quantitative and two qualitative transitions were generated for each analyte. The dwell times were adjusted to deliver about 4 Hz acquisition frequency.

Study Sequence: A 5 day long evaluation period was designed to determine the system precision/robustness. Each day the following sequence was executed:

Daily Injections (Repeated for 5 days)		
1	Blank	Toluene
2-11 (10)	Calibration	0, 1, 2.5, 5, 10, 20, 30, 50, 80, 100 ng/ml
	equivalent to	0, 0.2, 0.5, 1, 2, 4 ,6, 10, 16, 20 ug/kg in matrix
12	Blank	Toluene
13-18 (6)	QC sample	1 ng/ml, equivalent to 0.2 µg/kg in matrix
19	Blank	Toluene
20-25 (6)	Palm oil extracts	5 ng/ml, equivalent to 1 µg/kg in matrix
26	Blank	Toluene
27-32 (6)	QC sample	1 ng/ml, equivalent to 0.2 µg/kg in matrix
33	Blank	Toluene
34-39 (6)	Palm oil extracts	5 ng/ml, equivalent to 1 µg/kg in matrix
40	Blank	Toluene

Results and Discussion

Calibration results



A 10 point linear calibration curve was prepared daily. Excellent linearity was obtained and the calibration curve generated on the first day was virtually identical to the one generated on the 5th day. The R^2 difference was only 0.0004, indicating that the system conditions remained unchanged despite the injection of over hundred samples in between, many of them the palm oil extract with heavy matrix.

The blue triangles show the results of the 12 QC samples, while the red triangles identify the 12 palm oil extract results. As the graphs show, exceptional precision and accuracy is exhibited both on the first and on the last day. Accuracy of the measured Benzo(a)pyrene concentration in matrix ranged from 93% - 101% during the 5 day period.

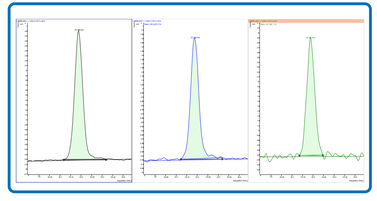
The area count for each of the PAHs detected in the palm oil extract for 12 injections is shown below for day 1 and day 5. The stable response resulted very low area count %RSDs for each analyte every day. Even the combined day 1 and day 5 results deliver %RSD below 4%. Note that this is based just on raw area count, without using the internal standard response to correct for small operational imprecisions often encountered when analyzing a complex matrix.

Precision and accuracy

Spiked Palm Oil Areas	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
Sample	BaA		Chrysene		BbF		BaP	
SPK_OIL-1	124833	125119	119104	118308	149500	147912	167868	154471
SPK_OIL-2	122837	132562	116891	127786	148031	158223	171496	185316
SPK_OIL-3	126858	120574	118272	109267	152958	144451	174546	162590
SPK_OIL-4	124750	126248	119199	122896	147486	149448	166499	172664
SPK_OIL-5	126454	128350	120454	118989	151083	149821	174304	170538
SPK_OIL-6	125048	124918	117413	116110	146604	147202	169356	160305
SPK_OIL-7	126848	127236	120370	121535	155079	149775	168295	169821
SPK_OIL-8	128167	133703	120799	128893	150774	158544	174729	182656
SPK_OIL-9	121409	121916	117578	115348	151576	146707	168768	165262
SPK_OIL-10	122218	125474	118858	124321	149693	148796	170291	166748
SPK_OIL-11	125949	128717	120147	122050	151454	153817	175673	166051
SPK_OIL-12	129523	127455	121779	121687	156374	149013	172214	170050
%RSD Area (12 inj.)	1.8	2.9	1.2	4.3	1.9	2.8	1.7	4.9
&RSD Area (24 inj.- day1 + day5)	2.5		3.2		2.4		3.7	

Results and Discussion

Detection levels



The chromatograms represent the quant and qualifier ion plots of Benzo(a)pyrene at the lowest calibration level, at 1 ng/ml, or 1 pg on column. The JetClean source also assured that the chromatographic peak shapes remained Gaussian from day 1 to day 5, delivering strong, easily quantifiable peaks. The 1 pg amount corresponds to 5 times lower concentration than the maximum allowed amount in sample extracts, even in the most demanding matrix such as baby food, easily meeting and exceeding the regulatory requirements.

The precision and accuracy derived from multiple injections of the QC sample (= 1 ng/ml) is as remarkably good as the results shown in the table for the palm oil extract.
The standard deviation (n=8) of the detected amount for benzo(a)pyrene was 0.0582, resulting in a statistically derived (99% conf. level, n-1 degrees of freedom) detection limit of 0.175pg.
This measurements should be repeated, as most likely the concentration used for the multiple injections is too high, resulting in an estimated detection limit much higher than the system can actually achieve.

Cleaning Frequency A system with the same configuration was recently deployed in a food laboratory. It has delivered similarly remarkable results and uninterrupted operation since installation, now 11+ months ago, eliminating the monthly manual cleaning previously needed. The reduced source cleaning frequency provides both increased productivity and convenience. The successful detection of PAHs in environmental samples without manual cleaning for months was also reported[1].

With JetClean Self-cleaning ion source

July	Aug	Sept
Oct	Nov	Dec
Jan	Febr	March
April	May	June

Without JetClean Self-cleaning ion source

July	Aug	Sept
Oct	Nov	Dec
Jan	Febr	March
April	May	June

Conclusions

The system accuracy, precision and robustness was demonstrated in the 5 day long laboratory test with exceptional results. The GC/TQ equipped with a JetClean source makes it possible to comply easily with the EU regulations in food, even in infant formulas. It delivers detection limits more than 5 times lower than the regulated maximum levels along with outstanding precision and accuracy for extended periods of time. Subsequent field deployment of a similarly configured system 11 months ago in a food laboratory delivered equally outstanding results,

Reference

Anderson, Kim A., et al. "Modified ion source triple quadrupole mass spectrometer gas chromatograph for polycyclic aromatic hydrocarbon analyses." *Journal of Chromatography A* 1419 (2015): 89-98.