

Determination of Benzo[a]pyrene and Benz[a]anthracene in Coal Tar and Pitch Products by Automated SPE-GC/MS

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KEYWORDS

Coal Tar, Pitch, Benzo[a]pyrene, Benz[a]anthracene, Polycyclic Aromatic Hydrocarbons (PAH), Solid Phase Extraction (SPE), Automation, GC/MS

ABSTRACT

This note presents the automated determination of benzo[a]pyrene and benz[a]anthracene in tar distillation products by SPE-GC/MS. Samples are dissolved in tetrahydrofuran (THF), placed in sample vials and internal standard added. The vials are then placed in the autosampler. All further sample preparation steps which include SPE cleanup on silica gel cartridges and injection into the GC/MS are performed automatically. Eight replicates of each sample were analyzed resulting in relative standard deviations between 0.8 and 2.6 %. Analysis results were compared with results of an LC reference method with fluorescence detection [1]. Both methods gave equivalent results with maximum deviations of +6.7 and -11.3 % for benzo[a]pyrene.

These data show that the developed SPE-GC/MS analysis method is precise and accurate. The SPE system performs sample preparation during the chromatographic run of the preceding sample for maximum throughput, enabling the analysis of around 40 samples per day.

INTRODUCTION

Distillation of coal tar is performed to produce chemical compounds for industrial use. The resulting products are viscous black liquids or black solids depending on the fraction collected. These products are used in road construction, for impregnation and sealing, as binder in electrode and refractory products etc.

As part of the quality control process, concentrations of benzo[a]pyrene and in some products benz[a]anthracene need to be determined. Expected values for benzo[a]pyrene are in the range from approximately 10 to 1000 mg/kg (ppm), for benz[a]anthracene around 20 mg/kg. The analysis can be performed by LC with fluorescence detection (FLD) following a standard method [1]. Generally it is quite challenging to perform the analysis due to the high levels of matrix compounds, which can cause interferences, signal suppression and contamination of the analysis system. This document describes an alternative analysis method based on automated SPE-GC/MS. Sample fractionation and cleanup on silica gel cartridges helps to keep the GC inlet liner, column and MS clean enabling extended maintenance intervals.

EXPERIMENTAL

Instrumentation. Sample preparation was performed on a MultiPurpose Sampler (MPS), Dual Head version equipped with a Solid Phase Extraction (SPE) module (both GERSTEL) and mounted on a GC/MS system (figure 1).



Figure 1. GC/MS system used for automated determination of benzo[a]pyrene and benz[a]anthracene in tar distillation products.

The autosampler tower (head) to the right was equipped with a 5 mL syringe used for all sample preparation steps; the left tower was equipped with a 10 μ L syringe used for injection into the GC. All solvents and samples were delivered by the 5 mL syringe providing a controlled and repeatable flow across the SPE cartridge. Standard SPE cartridges cut off at the top and equipped with transport adapters and syringe needles were used for extraction (figure 2). Such cartridges are commercially available from a number of vendors: Macherey-Nagel, Sigma-Aldrich, Bekolot and others. The complete process is conveniently controlled using the GERSTEL MAESTRO software.



Figure 2. Top: Standard solid phase extraction (SPE) cartridge adapted for automated SPE with the GERSTEL MPS. Such cartridges are commercially available from a number of vendors. Bottom: Standard solid phase extraction cartridge.

The overall workflow is depicted in figure 3. Sealed vials and cartridges are used to minimize the risk of sample contamination and solvent loss.

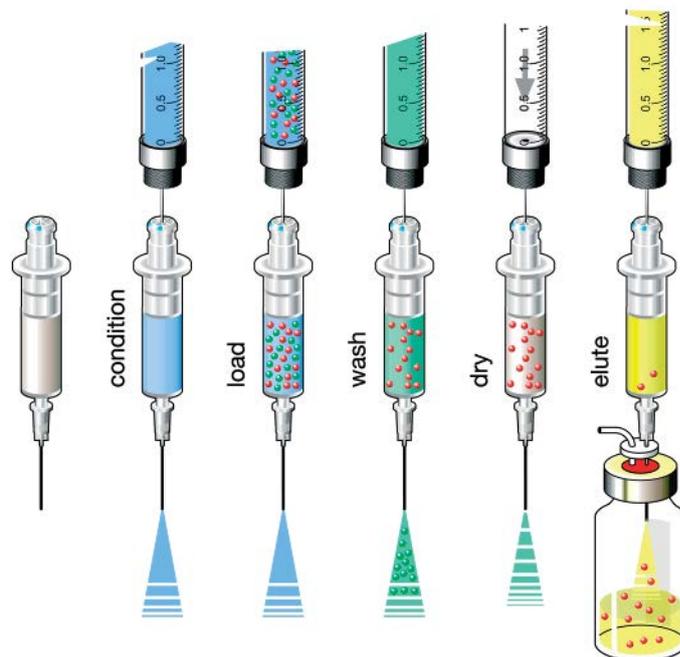


Figure 3. Workflow of automated SPE with the GERSTEL SPE module.

The MPS was mounted on a 7890 GC coupled to a 5977 MSD (both Agilent Technologies) for GC/MS analysis. Sample introduction was done via a Cooled Injection System CIS 6 PTV type GC inlet (GERSTEL). The analytical column used was an Rxi-5ms 30 m, $d_i = 0.25$ mm, $d_f = 0.25$ μ m (Restek).

Materials and Solvents. Five different tar based samples were analyzed:

Sample A: Pitch product - binder for the production of refractory bricks

Sample B: Pitch product - binder for refractory products

Sample C: Coal tar product

Sample D: Coal tar product

Sample E: Anthracene oil

Samples were cleaned up on 6 mL SiOH (1000 mg) SPE cartridges (Macherey-Nagel, 730075MPS). These were heated at 110°C before use to remove adsorbed water and stored over a drying agent. These SPE cartridges can be purchased equipped with transport adapter and syringe needle facilitating the SPE automation. All solvents were of analytical grade.

Preparation of Calibration Standards and Sample Solutions. Solutions of the internal standards benzo[a]pyrene-d₁₂ and benz[a]anthracene-d₁₂ were prepared by weighing in exact amounts of both compounds and filling up to 1000 mL with tetrahydrofuran (THF). This resulted in a concentration of 9.9 ng/μL for benzo[a]pyrene-d₁₂ and 10.0 ng/μL for benz[a]anthracene-d₁₂.

Calibration standards were prepared by mixing a certified reference PAH standard in acetonitrile (NIST 1647d) with both internal standard solutions and acetonitrile according to table 1. Calibration standards were prepared automatically using the MultiPurpose Sampler (MPS) resulting in high precision and accuracy.

Table 1. Calibration solutions for the quantification of benzo[a]pyrene and benz[a]anthracene.

Level	Volume NIST 1647d standard [μL]	Volume benzo[a]pyrene-d ₁₂ solution [μL]	Volume benz[a]anthracene-d ₁₂ solution [μL]	Volume acetonitrile [μL]	Total volume [μL]	Concentration benzo[a]pyrene [pg/μL]	Concentration benz[a]anthracene [pg/μL]	Concentration benzo[a]pyrene-d ₁₂ [pg/μL]	Concentration benz[a]anthracene-d ₁₂ [pg/μL]
Cal 1	5	60	60	7795	7920	3.10	2.58	75.4	75.9
Cal 2	10	30	30	3890	3960	12.4	10.3	75.4	75.9
Cal 3	10	10	10	1290	1320	37.2	31.0	75.4	75.9
Cal 4	15	10	10	1285	1320	55.8	46.5	75.4	75.9
Cal 5	20	10	10	1280	1320	74.4	62.0	75.4	75.9
Cal 6	30	10	10	1270	1320	111	93.0	75.4	75.9
Cal 7	50	10	10	1250	1320	186	155	75.4	75.9

Samples were weighed in as listed in table 2. Each 5 mL of the benzo[a]pyrene-d₁₂ solution and benz[a]anthracene-d₁₂ solution (only for Sample E) were added and the mixture was filled up to 50 mL with tetrahydrofuran.

Table 2. Preparation of sample solutions.

Sample	Sample weight [mg]	Sample volume [mL]	Sample concentration [mg/mL]	Sample volume injected into SPE [mL]	Eluate volume* [mL]
A	151.8	50	3.036	0.6	7.92
B	204.7	50	4.094	0.6	7.92
C	31.8	50	0.636	0.6	7.92
D	2494.3	50	49.886	0.6	7.92
E	1994.7	50	39.894	0.6	7.92

*The eluate volume was determined experimentally.

The resulting concentration of both internal standards in the eluate after SPE cleanup was around 75 pg/μL, which corresponds well with the concentration of the internal standards in the calibration solutions. Calibration was done by liquid injection of the described solvent-based standards without SPE cleanup.

Sample Preparation - Manual Steps

- Put around 1.5 mL of each sample solution into a 2 mL vial and position the closed vial on the MPS.

Sample Preparation - Automated Steps

- Condition the SPE cartridge with 8 mL of acetonitrile.
- Load 0.6 mL sample onto the SPE cartridge.
- Elute the analytes with 8 mL acetonitrile.

Analysis Conditions

MPS:		MSD:	
Syringe	10 μ L	Mode	Selected ion monitoring (SIM)
Injection Volume	1.5 μ L	Source Temperature	320 $^{\circ}$ C
CIS:		Quad Temperature	180 $^{\circ}$ C
Temperature	40 $^{\circ}$ C; 12 $^{\circ}$ C/s; 350 $^{\circ}$ C (15 min)	SIM Masses	
Pneumatics	Splitless, 5 min	Benzo[a]pyrene	m/z 252/250/126
Liner	Glass beads (011714-005-00)	Benzo[a]pyrene-d ₁₂	m/z 264/260/132
GC:		Benz[a]anthracene	m/z 228/229/226
Pneumatics	1.2 mL/min He, constant flow	Benzo[a]anthracene-d ₁₂	m/z 240/241/236
Oven	45 $^{\circ}$ C (1 min); 10 $^{\circ}$ C/min; 325 $^{\circ}$ C (1 min)	Resolution high;	
Column	30 m Rxi-5ms (Restek)	dwelt time	40 ms for quantifier, 30 ms for qualifier
	d _i = 0.25 mm, d _f = 0.25 μ m		
Transfer Line	320 $^{\circ}$ C		

RESULTS AND DISCUSSION

In order to test the cleanup on the silica gel SPE cartridge, a fractionated elution of Sample B with each 1 mL of solvent was performed (figure 4). A good cleanup effect was visible, as can be seen from the clean full scan chromatogram of Sample A in figure 5. The background is relatively low especially in the elution range of benz[a]anthracene and benzo[a]pyrene. Analyte and internal standard peaks were well separated from background signals, see figures 6-11. Although the samples were cleaned up quite well, internal standards were necessary to compensate for matrix effects and to get an accurate quantitation.



Figure 4. Fractionated elution of sample B loaded onto a silica gel SPE cartridge. The cleanup effect is clearly visible.

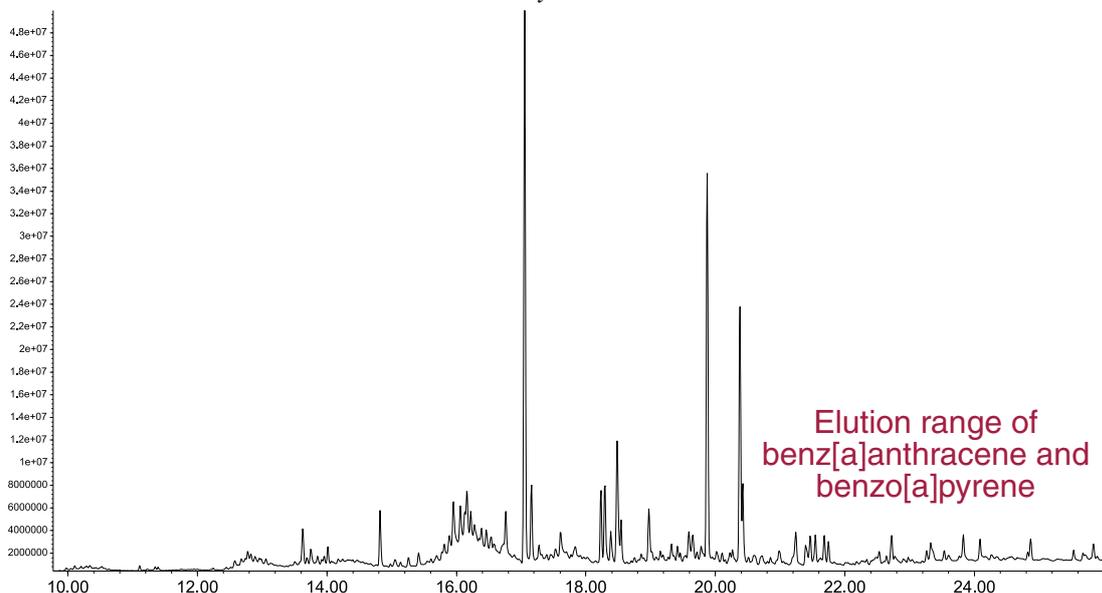


Figure 5. Example: Full scan chromatogram of sample A after SPE cleanup.

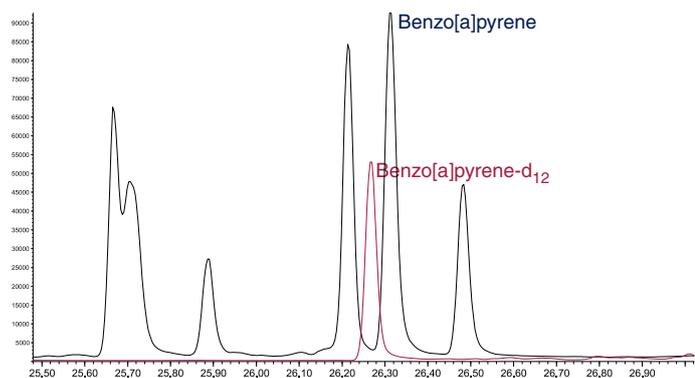


Figure 6. Benzo[a]pyrene in sample A, SIM, m/z 252; 264.

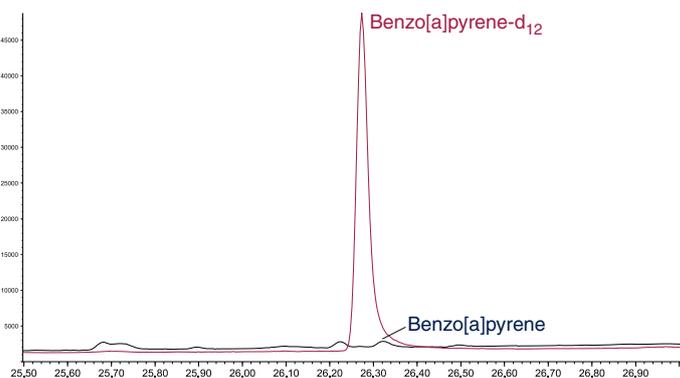


Figure 10. Benzo[a]pyrene in sample E, SIM, m/z 252; 264.

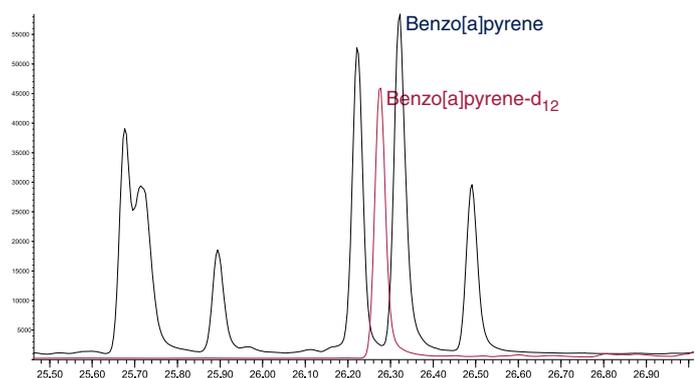


Figure 7. Benzo[a]pyrene in sample B, SIM, m/z 252; 264.

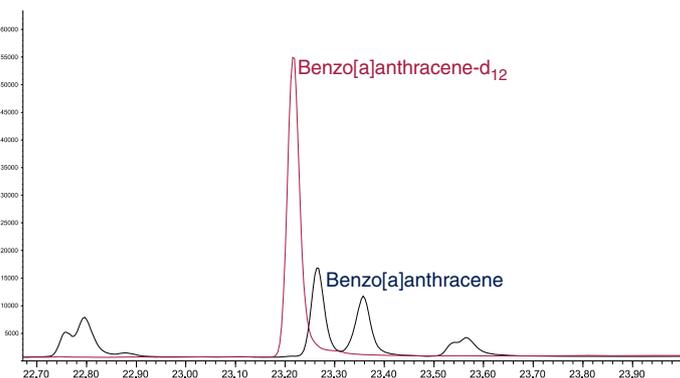


Figure 11. Benzo[a]anthracene in sample E, SIM, m/z 228; 240.

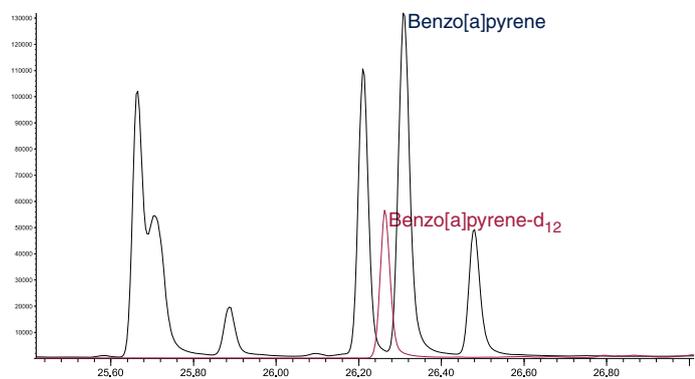


Figure 8. Benzo[a]pyrene in sample C, SIM, m/z 252; 264.

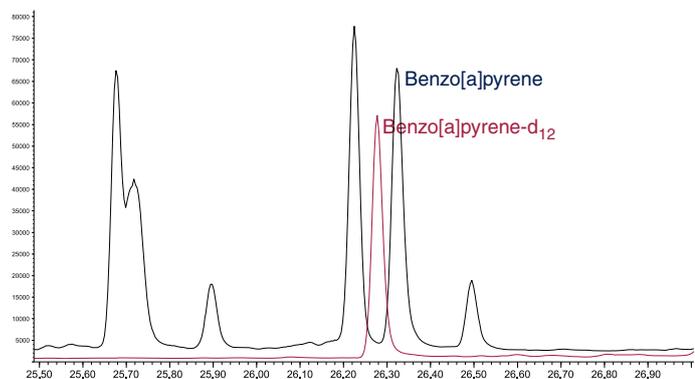


Figure 9. Benzo[a]pyrene in sample D, SIM, m/z 252; 264.

Five different types of samples were analyzed repeatedly (8 times) in order to demonstrate the robustness of the developed method and the instrumentation used (details below). Analysis values were compared with the results of the LC/FLD reference method (LC/FLD conducted at customer's site). A calibration with standards, including the internal standards, generated in pure solvent was conducted for both analytes (figure 12 and 13). The original sample solutions, SPE cartridges and resulting eluates can be seen in figure 14.

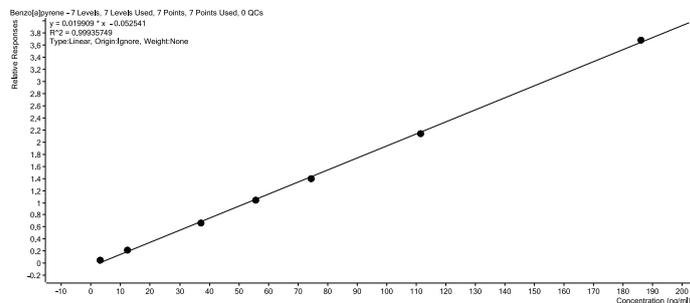


Figure 12. Calibration curve for benzo[a]pyrene, 3.1-186 ng/mL.

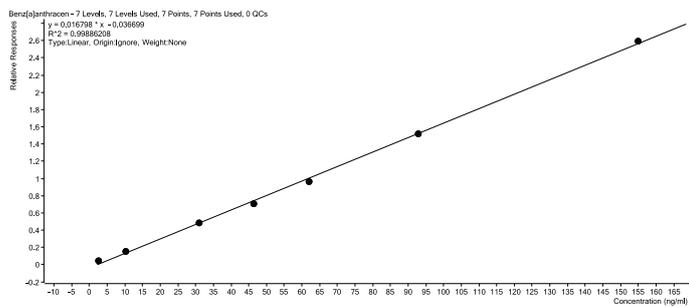


Figure 13. Calibration curve for benz[a]anthracene, 2.6-155 ng/mL.

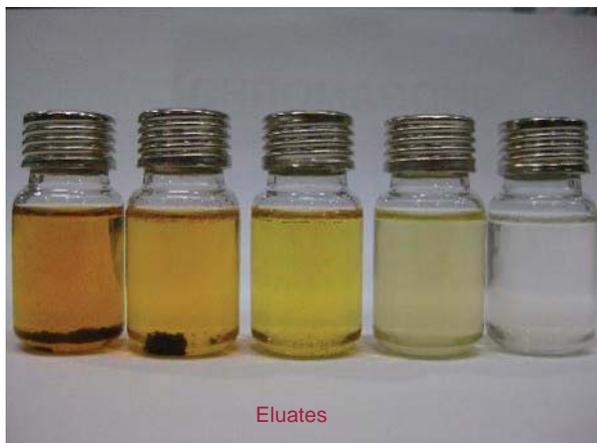


Figure 14. Top: Original sample solutions. Middle: SPE cartridges after use. Bottom: Eluates. Samples from left to right: A, B, C, D and E.

Analysis values for benzo[a]pyrene were calculated according to the formula in figure 15 and showed good agreement (between +6.7 and -11.3 % for benzo[a]pyrene) with the values determined by the LC/FLD method (table 3). The largest difference was found for benz[a]anthracene in anthracene oil. For this analyte the LC/FLD method was not completely optimized resulting in too high analysis values. The repeatability of the developed method was excellent with relative standard deviations (RSDs) of absolute peak areas for both analytes and internal standards between 1.1 and 11.4 % (for examples, see tables 4 and 5). Relative standard deviations for analysis values were between 0.8 and 2.6 % (table 3) demonstrating the high precision of the method.

$$\text{Analysis value} \left[\frac{\text{mg}}{\text{kg}} \right] = \frac{\text{Concentration in eluate} \left[\frac{\text{ng}}{\text{mL}} \right] \cdot \text{Eluate volume} [\text{mL}] \cdot \text{Sample volume} [\text{mL}]}{\text{Sample volume injected into SPE} [\text{mL}] \cdot \text{Sample weight} [\text{mg}]}$$

Figure 15. Formula for calculating analysis values.

Table 3. Analysis values and relative standard deviations for different tar distillation products. Repeatabilities and analysis values were calculated from 8 replicate analyses (complete SPE-GC/MS workflow).

Sample and analyte	Repeatability SPE-GC/MS RSD [%]	Analysis value SPE-GC/MS [mg/kg]	Analysis value LC/FLD [mg/kg]
A benzo[a]pyrene	1.5	411	413
B benzo[a]pyrene	0.8	226	217
C benzo[a]pyrene	1.1	2791	3105
D benzo[a]pyrene	1.6	17	16
E benzo[a]pyrene	2.6	1.2	<20
E benz[a]anthracene	2.0	6.6	26

Table 4. Peak areas from replicate analyses (complete SPE-GC/MS workflow) of sample A.

Sample	Peak area benzo[a]pyrene	Peak area benzo[a]pyrene-d ₁₂	Peak area benz[a]anthracene	Peak area benz[a]anthracene-d ₁₂
Sample A, 1	119557	89467	99641	N/A
Sample A, 2	119235	87711	104777	N/A
Sample A, 3	118082	88502	103307	N/A
Sample A, 4	115506	86074	93944	N/A
Sample A, 5	118003	87055	105633	N/A
Sample A, 6	116768	87628	105262	N/A
Sample A, 7	117380	88627	103254	N/A
Sample A, 8	118863	88381	102542	N/A
Average	117924	87931	102295	N/A
RSD [%]	1.1	1.2	3.8	N/A

Table 5. Peak areas from replicate analyses (complete SPE-GC/MS workflow) of sample E.

Sample	Peak area benzo[a]pyrene	Peak area benzo[a]pyrene-d ₁₂	Peak area benz[a]anthracene	Peak area benz[a]anthracene-d ₁₂
Sample E, 1	2068	94226	29967	99096
Sample E, 2	1788	99819	32132	104494
Sample E, 3	1643	91228	28585	94965
Sample E, 4	1708	90828	28432	96208
Sample E, 5	1465	85542	25768	88963
Sample E, 6	1553	94615	29150	98857
Sample E, 7	1516	94294	28973	99535
Sample E, 8	1664	92371	28330	98326
Average	1676	92865	28917	97556
RSD [%]	11.4	4.4	6.1	4.6

The sample cleanup helps to keep inlet liner, column and the MS system clean, enabling extended maintenance intervals. Please refer to figure 16 for a comparison of the inlet liner before and after analysis of more than 50 samples and figure 17 for a comparison of peak areas in a calibration standard before and after sample analysis. It was later determined that elution with toluene rather than acetonitrile results in even cleaner chromatograms (figure 18).



Figure 16. CIS GC inlet liner new (top) and after >50 samples (bottom). Due to effective sample cleanup, the used inlet liner is relatively clean.

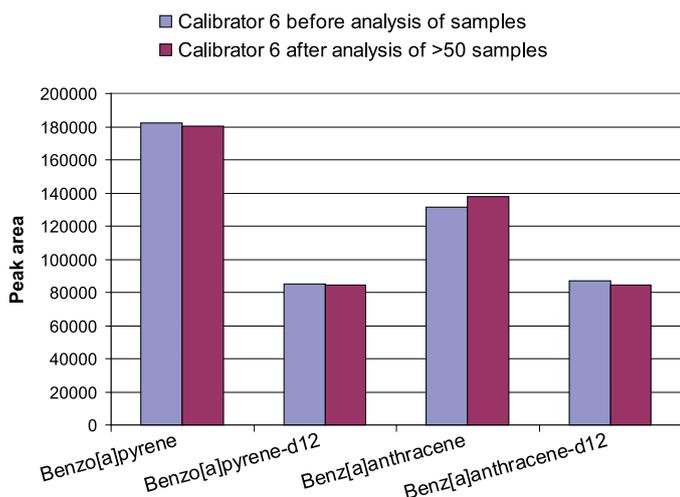
Calibrator before and after sample analysis

Figure 17. Peak areas for calibration standard 6 remain constant even after analysis of more than 50 samples.

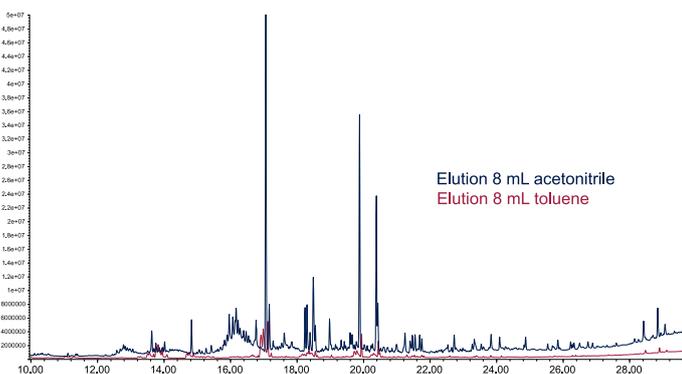


Figure 18. Comparison between elution of the silica gel SPE cartridge with acetonitrile (as done in this work) and with toluene - Full scan chromatograms of sample A. The use of toluene results in a better sample cleanup and could further improve the method.

The GERSTEL MAESTRO software integrated in the Agilent ChemStation or MassHunter controls the complete sample preparation process. Automated overlapping of sample preparation and chromatographic analysis enables the GC/MS-system to operate at full capacity since the next sample is always prepared and ready to be introduced whenever the GC/MS run has been completed. With a GC runtime of 30 min and adding a 5 min cooldown time, a total throughput of around 40 samples per day is possible (not including calibration, quality control standards and blanks).

CONCLUSIONS

An SPE-GC/MS analysis method for the determination of benzo[a]pyrene and benz[a]anthracene in different tar distillation products was developed.

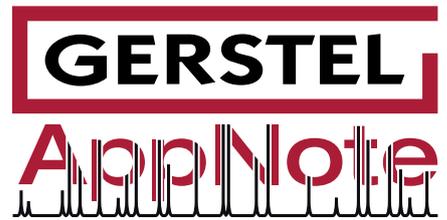
The sample is dissolved in THF, internal standards are added (benzo[a]pyrene-d₁₂ and benz[a]anthracene-d₁₂), and the solution is transferred to a vial, which is placed in the autosampler tray. From that point on, the process is fully automated. The sample is cleaned up using silica gel SPE cartridges (6 mL / 1 g) and an aliquot is injected into the GC/MS system. Manual sample preparation normally represents a significant workload for laboratory staff and it entails exposure to potentially toxic solvents and reagents. Furthermore, in manual processes, errors are more likely to occur as are operator to operator variations. Therefore, automation of the analysis is often preferable.

Analysis results achieved based on the automated SPE-GC/MS method are precise with relative standard deviations between 0.8 and 2.6 %. Further, they are in good agreement with the LC/FLD reference analysis method. A sample throughput of 40 samples per day can be achieved. Calibration solutions were automatically prepared by the MPS resulting in high precision and accuracy.

The analysis system is highly flexible and opens the possibility of automating further SPE methods. If additional modules are configured, many other types of sample preparation workflows can be automated as well.

REFERENCES

- [1] DIN EN 1014-3:2010-11, Wood preservatives - Creosote and creosoted timber - Methods of sampling and analysis - Part 3: Determination of the benzo[a]pyrene content of creosote



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