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APPLICATIONS

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Ramkumar loves to write poems, and to watch & read Shakespeare's plays.

Polycyclic Aromatic Hydrocarbons (PAHs) in Tattoo Ink by GC/MS

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Polycyclic aromatic hydrocarbons (PAHs) have been found in tattoo inks at significantly high levels that can range from 0.14 to 201 µg/g)[1]. Because tattoo inks are injected directly into the skin, the PAH content causes a potential health risk. This technical note presents a GC method for PAH analysis following a basic LLE sample preparation. PAH recovery decreased with increasing molecular weight due to absorbance with carbon-black pigments in the tattoo ink; quantitation using a deuterated internal standard is therefore recommended.

Introduction

Tattoos have been popular in cultures around the world for centuries. In recent years, however, modern ink has come under scrutiny due to health concerns related to the purity of its contents. Polycyclic aromatic hydrocarbons (PAHs) in particular are listed as human carcinogens by the International Agency for Research on Cancer (IARC) and can form during ink production. Additionally, tattoo ink contains azo dyes that can form byproducts or break down into hazardous substances that cause allergic reactions or hypersensitivity; black and red dyes in particular are usually polyaromatic azo dyes, which can break down into individual polyaromatic amines.

In the present study, we explore methods for testing of impurities in tattoo ink. Techniques for preparation and extraction of analytes from tattoo dye followed by analysis by GC/MS are presented. PAH separations are conducted using a Zebron™ ZB-5MS*PLUS*™ GC column, which provided an inert phase for improved peak shapes. Following analysis, techniques for further optimizing GC method parameters are discussed.

Materials and Methods

Samples were prepared as shown in **Figure 1**. Following sample preparation, samples were analyzed by GC/MS; elution order, SIM ions for quantitation, and retention times for the analytes are listed in **Table 1**.

Figure 1.
Sample preparation protocol for PAH samples.

Weigh 1.0 g of black tattoo ink into a 5 mL centrifuge tube
↓
Spike in appropriate standards and deuterated internal standards
↓
Add 2.0 mL of ethyl acetate to centrifuge tube
↓
Shake the sample for 10 minutes using a mechanical shaker
↓
Remove 50 to 100 µL of supernatant and transfer to an autosampler vial for GC/MS analysis

Table 1. GC/MS conditions for analysis of tattoo ink samples.

Column:	Zebron ZB-5MS*PLUS*
Dimensions:	30 meter x 0.25 mm x 0.25 µm
Part No.:	7HG-G030-11
Guard Column:	10 m Z-Guard™ (7CG-G000-00-GZ0)
Injection:	Splitless @ 250 °C, 1.0 µL
Recommended Liner:	Zebron PLUS Single Taper Z-Liner™
Liner Part No.:	AG2-4B13-05 (for Shimadzu® Systems)
Carrier Gas:	Helium @ 1.4 mL/min (constant flow)
Oven Program:	75 °C for 1 min to 340 °C @ 20 °C/min for 2 min
Detector:	MSD @ 320 °C
Sample:	

Peak	Analyte	SIM Ions	RT (min)
1	1,4-Dichlorobenzene-d4	150,115	3.96
2	Naphthalene-d8	136,108	5.49
3	Naphthalene	128,129,127	5.52
4	2-Methylnaphthalene	142,141	6.54
5	Acenaphthylene	152,151,153	7.92
6	Acenaphthylene	162,80	8.14
7	Acenaphthene	154,153,152	8.19
8	Fluorene	166,165,167	9.05
9	Phenanthrene-d10	188,94	10.53
10	Phenanthrene	178,179,176	10.70
11	Anthracene	178,176,179	10.66
12	Fluoranthene	202,101,203	12.52
13	Pyrene	202,200,203	12.87
14	Benz[a]anthracene	228,229,226	14.81
15	Chrysene-d12	240,120	14.84
16	Chrysene	228,226,229	14.88
17	Benzo[b]fluoranthene	252,253,125	16.47
18	Benzo[k]fluoranthene	252,253,125	16.51
19	Benzo[a]pyrene	252,253,125	16.92
20	Perylene-d12	264,132	16.99
21	Indeno[1,2,3-cd]pyrene	276,138,227	18.35
22	Dibenzo[a,h]anthracene	278,139,279	18.38
23	Benzo[g,h,i]perylene	276,138,277	18.65

Results and Discussion

As shown in **Figures 2** and **3**, Zebron ZB-5MS*PLUS*™ provided good peak shape and resolution for the PAH analytes considered for this analysis. All analytes were chromatographically resolved from any matrix interferences with an analysis time of less than 20 minutes. As shown in **Figure 4**, analysis of a real tattoo ink sample revealed the presence of several PAHs.

Figures 5 and **6** show a strong correlation between molecular weight/elution order and recovery. As PAH molecular weight increases, so does the amount of absorption to carbon-black pigments in the tattoo ink. Black tattoo ink was the only matrix examined in this study, but the amount of recovery loss is expected to be less significant for non-black tattoo inks.

To account for recovery loss due to matrix absorption, deuterated internal standards (naphthalene-D8, acenaphthene-D10, phenanthrene-D10, chrysene-d12) were used for quantitation. A representative calibration curve using the internal standard method demonstrates good linearity and can be seen in **Figure 7**. As shown in **Table 2**, using the internal standard quantitation method resulted in acceptable accuracy and RSD values for a 10 µg/g spiked black tattoo ink sample.

Figure 2.
25 µg/mL calibration curve standard.

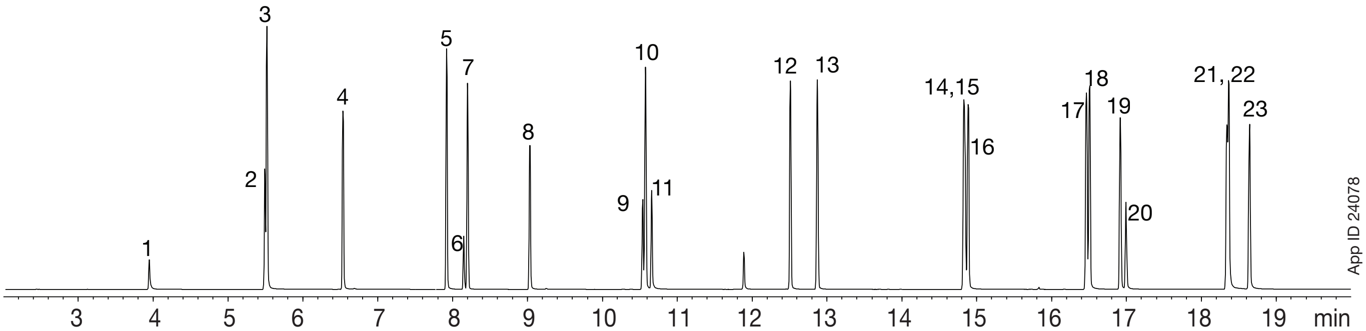

Figure 3.
Tattoo ink extract spiked at 10 µg/g.

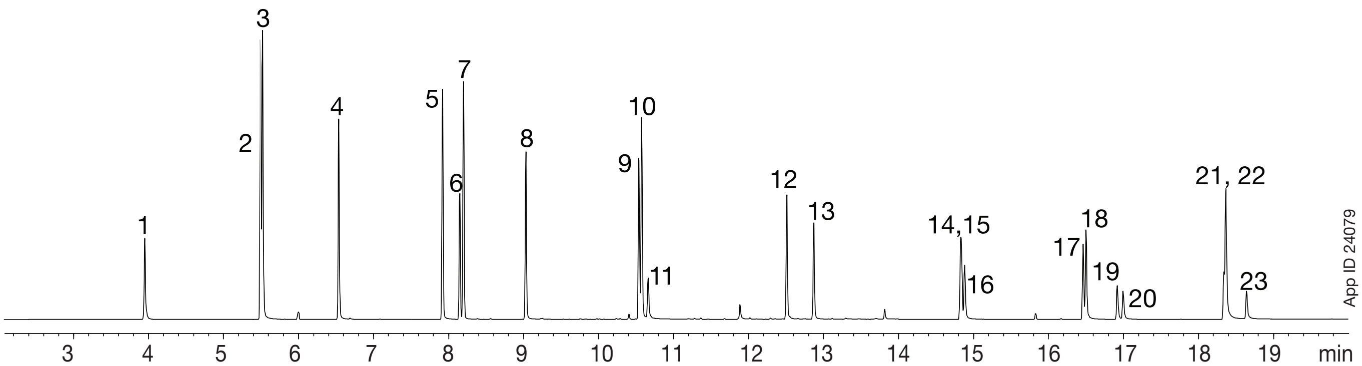

Figure 4.
Real sample extract of tattoo ink.

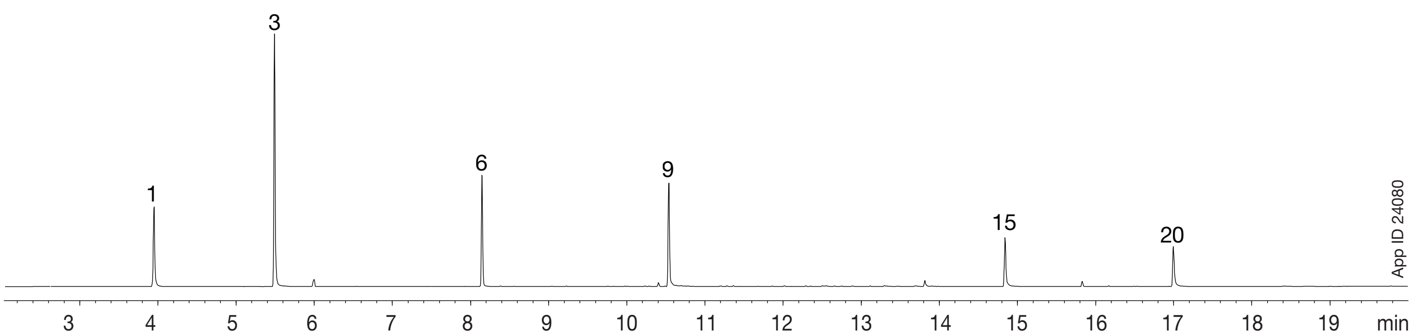

Figure 5.
Calculated concentration of tattoo ink extract (5 µg/g spike) without internal standard correction versus elution order.

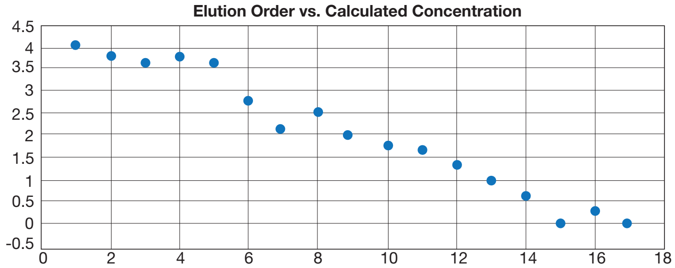

Figure 6.
Molecular weight of tattoo ink extract (5 µg/g spike) versus recovery.

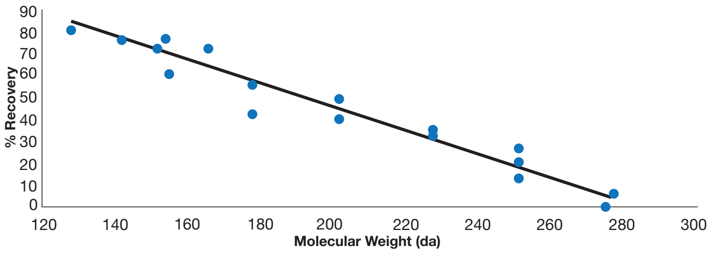

Figure 7.
Naphthalene calibration curve, 0.1 to 25 µg/mL.

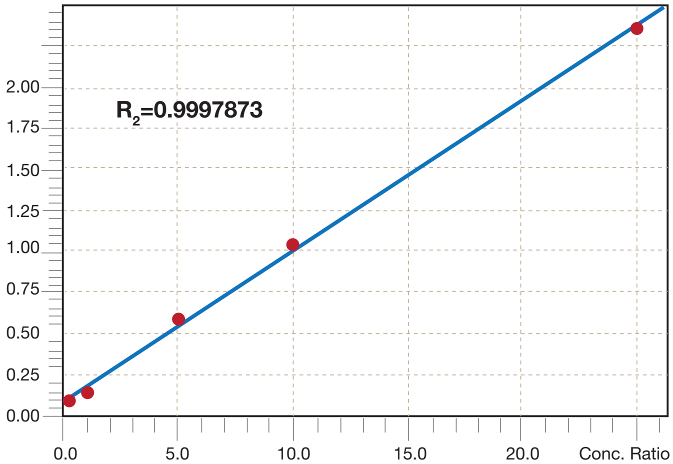

Table 2.
Recovery data for spiked tattoo ink extracts (10 µg/g).

Analyte	Calculated Concentration µg/g (n=3)	% Recovery	RSD (n=3)
Naphthalene	11.02	110	3.96
Acenaphthene	11.29	113	5.49
Phenanthrene	8.72	87	5.52
Chrysene	10.72	107	6.54

Conclusion

The Zebron ZB-5MS*PLUS*™ GC column provided good peak shape and resolution for PAHs extracted from tattoo ink. The simple LLE method provided acceptable accuracy and reproducibility when quantified with a deuterated internal standard. For GC/MS analysis of black tattoo inks, it is recommended that a deuterated analog be used as the internal standard for each analyte of interest.

References

1. Regensburger J., Lehner K., Maisch T., Vasold R., Santarelli F., Engel E., Gollmer A., Konig B., Landthaler M., and Baumler W. Tattoo inks contain polycyclic aromatic hydrocarbons that additionally generate deleterious singlet oxygen. Experimental Dermatology (2010). Volume 19: 275-281.

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Ordering Information

Zebron ZB-5MS*PLUS*™ GC Columns

Length (m)	ID (mm)	df (µm)	Temp. Limits (°C)	Part No.
15	0.25	0.25	-60 to 325/350	7EG-G030-11
20	0.18	0.18	-60 to 325/350	7FD-G030-08
20	0.18	0.36	-60 to 325/350	7FD-G030-53
30	0.25	0.25	-60 to 325/350	7HG-G030-11
30	0.25	0.50	-60 to 325/350	7HG-G030-17
30	0.25	1.00	-60 to 325/350	7HG-G030-22
30	0.32	0.25	-60 to 325/350	7HM-G030-11
30	0.32	1.00	-60 to 325/350	7HM-G030-22
60	0.25	0.25	-60 to 325/350	7KG-G030-11

Note: If you need a 5 in. cage, simply add a (-B) after the part number, e.g., 7HG-G030-11-B. Some exceptions may apply. Agilent 6850 and some SRI and process GC systems use only 5 in. cages.

Zebron PLUS GC Inlet Liners

Description	Dimensions ID x L (mm)	Part No.	Unit
For Shimadzu® 17A, 2014 and 2025 Models			
Single Taper Z-Liner™ 	3.4 x 95	AG2-3B13-05 AG2-3B13-25	5/pk 25/pk

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