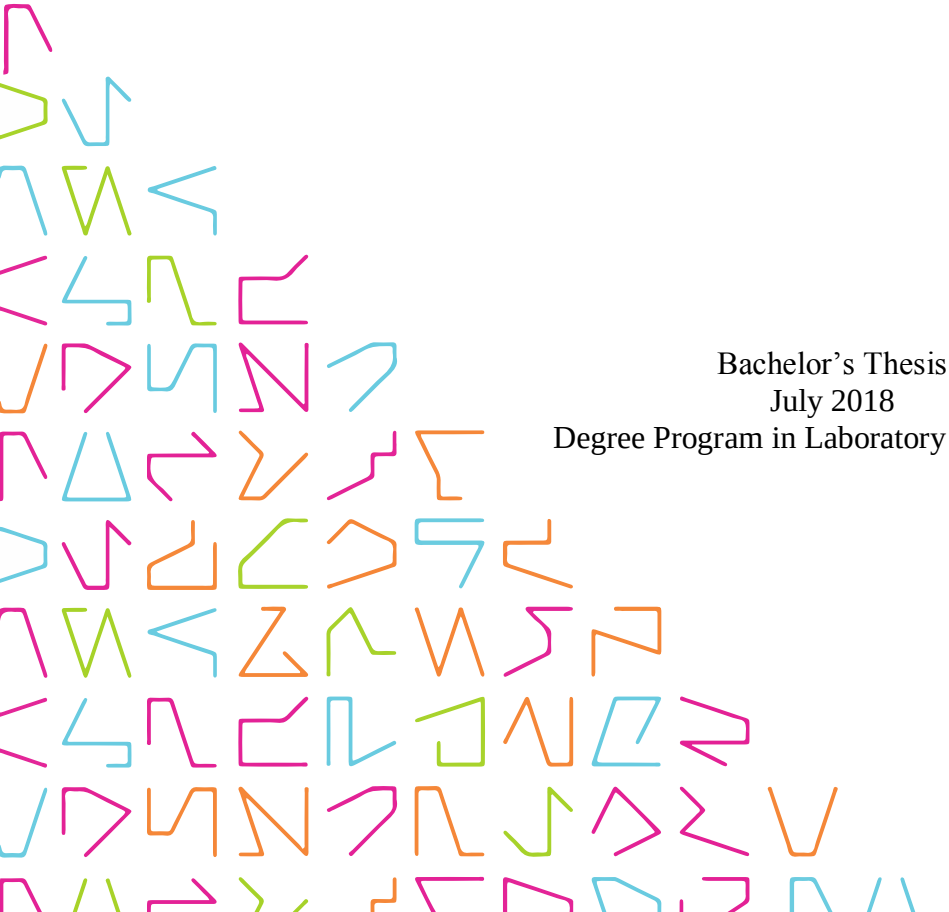
TAMPEREEN
AMMATTIKORKEAKOULU

ANALYSIS OF PAHs FROM SOIL AND SOLID WASTE WITH GC-MS

Method Development and Validation

Annika Rinne



Bachelor's Thesis
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ABSTRACT

Tampereen ammattikorkeakoulu
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RINNE, ANNIKA:
Analysis of PAHs from Soil and Solid Waste with GC-MS
Method Development and Validation

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This thesis was commissioned by the chemistry laboratory of Tampere University of Applied Sciences, which is a teaching laboratory that also provides selected analysis services for consumers. The laboratory wanted to be able to provide environmental analysis in the form of analysing polycyclic aromatic hydrocarbons from soil and solid waste, and a GC-MS instrument was provided for the analysis.

The aim was to provide the laboratory with an instrumental method capable of detecting PAHs, which are monitored from soil by the Government Decree 214/2007. The objective was to develop and validate the method using the equipment and chemicals provided by the laboratory. The method was based on SFS-EN 15527:2008 and SFS-ISO 18287:2006 standards and it was implemented for GC-MS instrument.

The sample preparation method and the instrumental method were finalized during this study. Instrumental limit of detection and limit of quantification were obtained during the validation process, but the values are not within the recommended values given in the standards the method is based on. This indicates a need for more thorough validation that includes the whole sample preparation procedure.

Key words: PAHs, validation, gas chromatography, mass spectrometry

TIIVISTELMÄ

Tampereen ammattikorkeakoulu
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Menetelmän kehitys ja validointi

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Opinnäytetyö tehtiin Tampereen ammattikorkeakoulun kemian laboratorioon, joka on opetuslaboratorio. Laboratorio tarjoaa myös valikoituja analyysejä asiakkailleen. Laboratorion haluttiin tarjoavan polysyklisten aromaattisten hiilivetyjen analysointia maasta ja kiinteästä jätteestä GC-MS laitteistolla.

Opinnäytetyön tavoitteena oli tuottaa laboratoriolle menetelmä, jolla voidaan analysoida PAH-yhdisteitä, joiden pitoisuutta maaperässä tarkkaillaan Valtioneuvoston asetuksen 214/2007 mukaisesti. Tarkoituksena oli kehittää ja validoida menetelmä SFS-EN 15527:2008 ja SFS-ISO 18287:2006 standardien pohjalta käyttäen laboratorion olemassa olevaa välinekantaa ja laitteistoja. Analyysissä käytettiin laboratorion GC-MS laitteistoa standardien suosituksen mukaan.

Näytteenkäsittely ja analyysimetodi saatiin lopullisiin muotoihinsa opinnäytetyön kokeellisen osion aikana. Validoinnista saatiin toteamis- ja määritysrajat laitteistolle, mutta nämä arvot olivat suurempia kuin standardeissa olevat raja-arvot, jonka perusteella kattavampi validointi on suositeltava jatkotoimenpide. Mikäli validointia jatketaan, toivotaan sen sisältävän myös näytteenkäsittelyn.

Asiasanat: PAH-yhdisteet, validointi, kaasukromatografia, massaspektrometria

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ABBREVATIONS AND TERMS

Calibration	in context of solutions, or calibration curve, sometimes called standard solutions or standard curve; exception with internal standard solution (ISTD)
EN	European standard; as approved by one of the European Standardization Organizations
GC-MS	Gas chromatograph – mass spectrometer
ISO	International Organization for Standardization
ISTD	Internal standard solution
LOD	Limit of Detection
LOQ	Limit of Quantification
PAHs	Polycyclic aromatic hydrocarbons
PIMA-Decree	Decree on the Assessment of Soil Contamination and Remediation Needs (214/2007) by the Ministry of Environment, Finland
SFS	Finnish Standards Association

In this report period (.) is used to indicate the decimal separation and a space ( ) is used to separate the thousands.

1 INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants, and their concentrations are monitored in soil and solid waste. In Finland the regulation of the compounds is based on the Government Decree on the Assessment of Soil Contamination and Remediation Needs (214/2007) by the Ministry of Environment, later referred in the text as PIMA-Decree, which gives guidelines on the values which PAHs can have in the soil before assessment and possible remediation are implemented. Analysing the PAH concentration from soil and solid waste of a site is therefore a necessary step before the verdict on how to proceed is given.

This study was conducted for the chemistry laboratory of Tampere University of Applied Sciences, which is a teaching laboratory that also provides selected analysis services. The aim was to provide the laboratory with an instrumental method capable of detecting PAHs. The objective was to develop the method and validate it for use of the laboratory.

The instrument used for the analysis is a gas chromatograph paired with a mass spectrometer detector. The sample preparation and instrumental validation were the focus of the experimental portion of this study.

2 COMPOUNDS

2.1 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) occur naturally in fossil fuels, but they are also formed in incomplete combustion of organic materials, for example in the burning of coal, oil, or from forest fires (Walker 2001, 165, 167.) PAHs are also produced by creosote, aluminium, and carbon anode manufacturing (Wright 2003, 340). PAHs born from combustion are first introduced to the environment as smoke particles or vapour, in which they appear as mixtures (Walker 2001, 169).

PAHs consist of two or more benzene rings linked together, but they may also include other ring structures, as evidenced by their chemical structures shown in figure 1 (Walker et al 2012, 8.) The chemical structures of all sixteen of the target PAHs are shown in figure 1, and the main properties are shown in table 1.

FIGURE 1. Chemical structures of target PAHs (PubChem Compound Database n.d.)

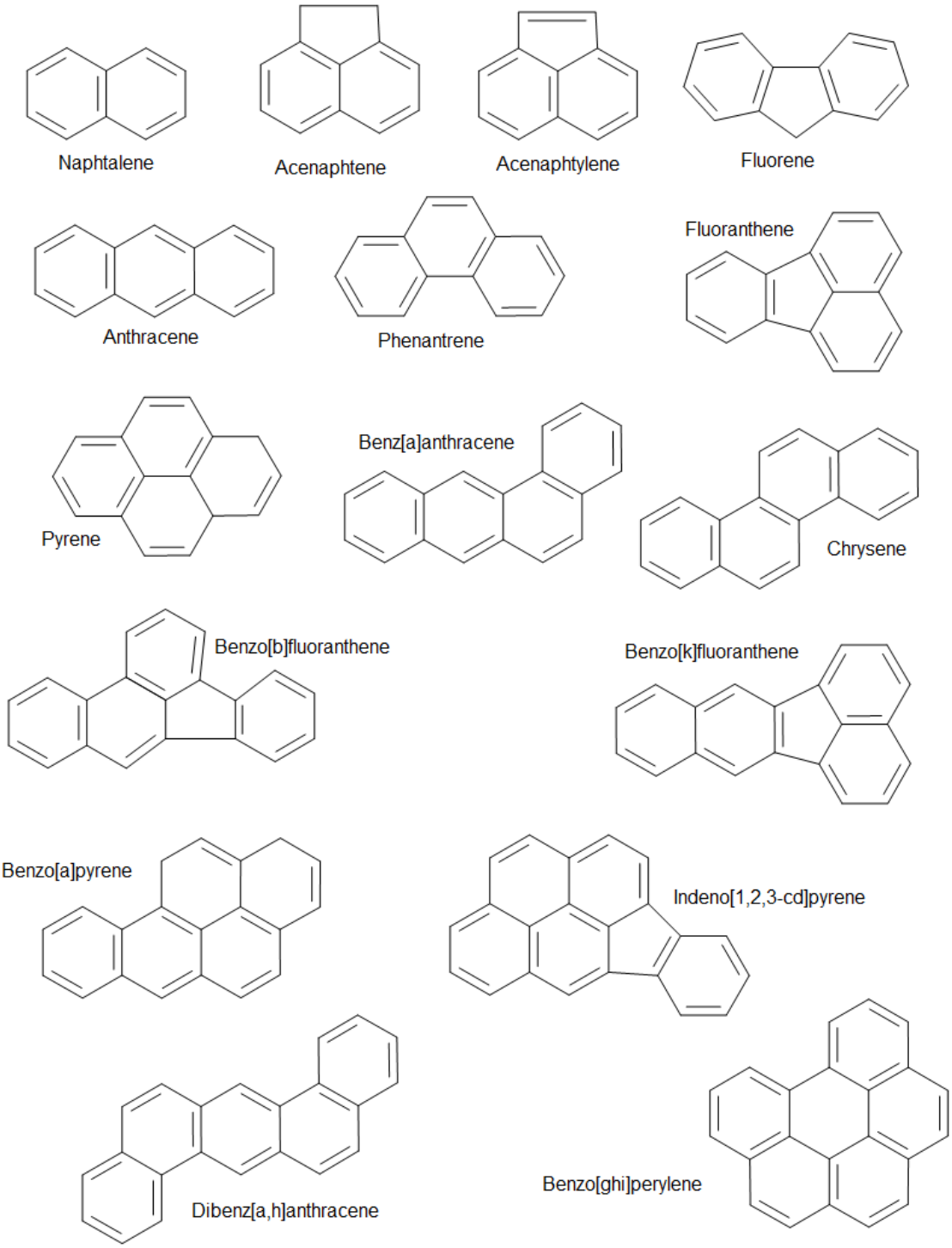

TABLE 1. Properties of the target PAHs (PubChem Compound Database n.d.)

Compound	**CAS-no**	**Chemical formula[2]**	**m/z**	**Boiling point °C**
Naphtalene	91-20-3	C_8H_{10}	128	218
Acenaphtene	83-32-9	$C_{12}H_{10}$	152	279
Acenaphtylene	208-96-8	$C_{12}H_8$	154	280
Fluorene	86-73-7	$C_{13}H_{10}$	166	298
Anthracene	120-17-7	$C_{14}H_{10}$	178	340
Phenanthrene	85-01-8	$C_{14}H_{10}$	178	340
Fluoranthene	206-44-0	$C_{16}H_{10}$	202	384
Pyrene	129-00-0	$C_{16}H_{10}$	202	
Benz[a]anthracene[1]	56-55-3	$C_{18}H_{12}$	228	437.6
Chrysene	218-01-9	$C_{18}H_{12}$	228	448
Benzo[b]fluoranthene[1]	205-99-2	$C_{20}H_{12}$	252	
Benzo[k]fluoranthene[1]	207-08-9	$C_{20}H_{12}$	252	
Benzo[a]pyrene[1]	50-32-8	$C_{20}H_{12}$	252	495
Indeno[1,2,3-cd]pyrene[1]	193-39-5	$C_{22}H_{12}$	276	
Dibenz[a,h]anhtracene[1]	53-70-3	$C_{22}H_{14}$	278	524
Benzo[ghi]perylene	191-24-2	$C_{22}H_{12}$	276	> 500

[1] International Agency for Research on Cancer, has identified as possible human carcinogen (Wright 2003, 339.)
[2] Chemical structures shown in figure 1

PAHs with more than four benzene rings condense from the air and become adsorbed onto the surfaces of suspended particles. This phenomenon is caused by their low vapour pressure, which also makes PAHs with two to four rings adsorb into particles during lower temperatures. (Baird & Cann 2008, 511–512.) PAHs have been classified as persistent organic pollutants as they have long half-lives and therefore do not degrade easily (Wright 2003, 339). They are also hydrophobic and insoluble in water, as well semi-volatile. (Wright 2003, 340; Baird & Cann 2008, 511–512.)

PAHs accumulate in plants and animals (Wright 2003, 339). According to Walker (2001, 171) PAHs appear not to express much toxicity in themselves, rather having toxic transformation products, while Wright (2003, 339) explicitly introduces all PAHs as toxic. The most potent PAH carcinogens are benz[a]anthracene, dibenz[ah]anthracene,

and benzo[a]pyrene (Wright 2003, 340). PAHs are not acutely toxic to terrestrial animals, but in the presence of UV light they can be toxic to fish (Walker 2001, 171). The most potently carcinogenic PAHs have a bay region, which is formed by the branching in the benzene ring sequence. The bay region is illustrated in figure 2 on benzo[a]pyrene. (Baird & Cann 2008, 513–515.)

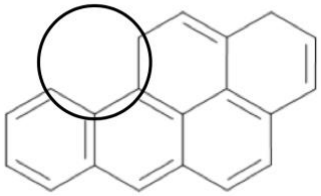

FIGURE 2. The bay region in benzo[a]pyrene (Baird & Cann 2008, 514)

2.2 Legislation

The Finnish legislation for assessing soil contamination and remediation was renewed in 2007 by the means of a government decree 214/2007, which states the following: "The assessment of soil contamination and remediation needs shall be based on an assessment of the hazard or harm to health or the environment represented by the harmful substances in the soil." The decree is not allowed to be applied to the assessment of sediment contamination and remediation. (Decree 214/2007, 1.)

The PIMA-Decree's appendices contain threshold and guideline values for some common harmful substances in soil as total concentration per dry matter. The decree contains such values for seven separate PAH compounds: anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(k)fluoranthene, phenanthrene, fluoranthene, and naphthalene; and values for total concentration of PAH compounds including the following compounds: anthracene, acenaphthene, acenaphthylene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, dibenzo(a,h)anthracene, phenanthrene, fluoranthene, fluorene, indeno(1,2,3-c,d)pyrene, chrysene, naphthalene, and pyrene. (Decree 214/2007, 4, 6.) The values relevant to PAHs given in the PIMA-Decree are shown in table 2.

TABLE 2. Threshold and guideline values for PAHs according to PIMA-Decree (Decree 214/2007, 4.)

Compound	**Threshold value mg/kg**	**Lower guideline value mg/kg**	**Higher guideline value mg/kg**
Naphthalene	1	5 (e)	15 (e)
Anthracene	1	5 (e)	15 (e)
Benz[a]anthracene	0.2	**2 (h)**	15 (e)
Benz[a]pyrene	1	5 (e)	15 (e)
Benz[k]fluoranthene	1	5 (e)	15 (e)
Phenanthrene	1	5 (e)	15 (e)
Fluoranthene	1	5 (e)	15 (e)
PAH[1]	15	30 (e)	100 (e)

[1] Total concentration of all target PAHs
In guideline values **(h) indicates health risks**, (e) indicates ecological risks

The threshold values indicate the need to assess the contamination and remediation needs of the soil if the values of one or more compound are over the value presented in the decree. In areas where the background concentration is higher than the threshold value, the background concentration indicates the need for assessment. If the values are over the higher guideline value in an industrial, storage or transport area, the soil is considered contaminated. The soil is also considered contaminated if the lower guideline value of one or more compounds is over the guidelines in any other areas not specified for higher guideline values. (Decree 214/2007, 3.)

The Finnish national standard and European standard 15527 (2008), later referred as SFS-EN 15527:2008, specifies the quantitative determination of 16 PAHs according to the priority list of the United States Environmental Protection Agency (US EPA, 1982). The standard is applicable for wastes such as contaminated soil, sludge and rubble, bitumen or waste containing bitumen. SFS-EN 15527:2008 describes a gas chromatographic method with mass spectrometric detection (GC-MS). The 16 PAHs specified in the standard are the same ones defined in PIMA-Decree's total PAH guidelines.

The Finnish national standard and International standard 18287 (2006), later referred as SFS-ISO 18287:2006, is based on International standard 13877 (1998) and is modified for the use of gas chromatographic method with mass spectrometric detection (GC-MS).

The standard presents two different extraction methods as well as specifies a quantitative determination of 16 PAHs according to the priority list of the United States Environmental Protection Agency (US EPA, 1982). SFS-ISO 18287:2006 is applicable to all types of soil (field-moist or chemically dried samples), and it covers a wide range of PAH contamination levels. The 16 PAHs specified in the standard are the same ones defined in PIMA-Decree's total PAH guidelines.

3 INSTRUMENTATION

3.1 Gas Chromatograph

Gas chromatograph (GC) has few requirements for the sample; it needs to be either gas or a volatile liquid. The sample is injected through a septum into an injection port from which the mobile phase carries it to the column. In gas chromatography the gaseous mobile phase is referred to as carrier gas, which is most often helium. The carrier gas carries the sample from the heated injector port to the column, which is either in temperature-controlled oven or is directly heated. The separation of the components happens in the column and heating the column during the analysis decreases the retention times of late-eluting components and sharpens peaks. The schematic diagram of a gas chromatograph is presented in figure 2. (Kellner, Mermer, Otto, Valcácel & Widmer 2004, 536, 539–540; Harris 2010, 565–566, 573–574.)

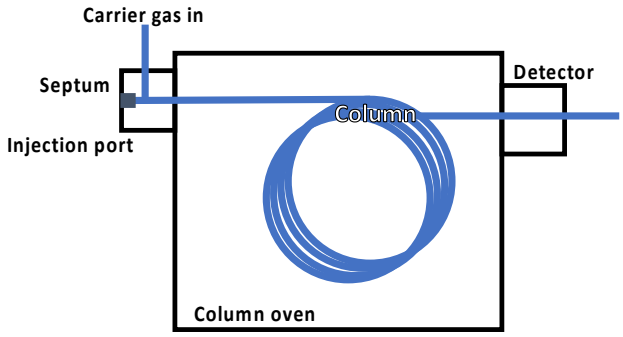

FIGURE 2. Schematic diagram of a gas chromatograph (Harris 2010, 566 Figure 23-1.)

The sample can be injected directly into the column (on-column injection) or more commonly through the injection port, which is heated and has a silanized glass liner. Injection is most often done with an autosampler. There are two different ways to do the injection through the port; split or splitless. A splitless is preferred when dealing with trace levels of high boiling components in low-boiling solvents, such as PAHs in n-hexane, but it is also suited for quantitative analysis. The main problems for splitless injection are matrix and memory effects, meaning some of the sample might not vaporize as it should, or some of the sample can remain in the injector and thus cause ghost peaks. (Kellner et al. 2004, 540, 542; Harris 2010, 577.)

Splitless injection injects the sample volume slowly into the liner with the split vent closed. Slow flow through the septum purge is maintained to remove any vapours that

escape from the injection liner. The injection temperature should be kept high enough for the analyte to be vaporous, but low enough to prevent decomposing. Some fractionation occurs during splitless injection, and about 80 % of the sample is applied to the column. (Kellner et al. 2004, 540, 542; Harris 2010, 578.)

Gas chromatographs usually use open tubular columns, which are long and thin. Their typical dimensions are 5 to 100 m in length, 0.1 to 0.53 mm in inner diameter, and 0.1 to 5 µm in stationary phase thickness. They are typically made of fused silica and coated with polyimide. Open tubular columns need only a small amount of sample (1 µl in final method), which means that sample preparation can be done in a smaller scale than if the required amount were larger. The performance of columns can weaken with age, or when exposed to temperatures that are too high. Columns typically have two temperature limits; a lower one within which the column can be held for a longer time, to for example condition the column and to bakeout the detector, and an upper one which the column can only be held at for a few minutes before it starts to decompose. This upper limit is suitable for quickly cleansing the column post run. (Kellner et al. 2004, 545; Harris 2010, 519, 566–568, 574.)

The oven is typically what heats up the column, but the column can have direct heating as well. The oven or heating is essential for temperature programming, which increases the vapour pressure of the sample and therefore decreases analysis time and sharpens peaks. (Harris 2010, 573–574.)

3.2 Mass Spectrometer

Mass spectrometry is a detection technique that studies the masses of atoms or fragments of molecules. Mass spectrometer (MS) is capable of forming a mass spectrum of a compound by ionizing the gaseous analyte, accelerating the ions by using an electric field, and separating the ions by their mass-to-charge ratio (*m/z*). Coupled with a chromatograph, mass spectrometer forms a chromatograph as well as a mass spectrum, which brings the retention time-based identification together with mass spectrum identification. Figure 3 shows a schematic diagram of a mass spectrometer. (Kellner et al. 2004, 824; Harris 2010, 502.)

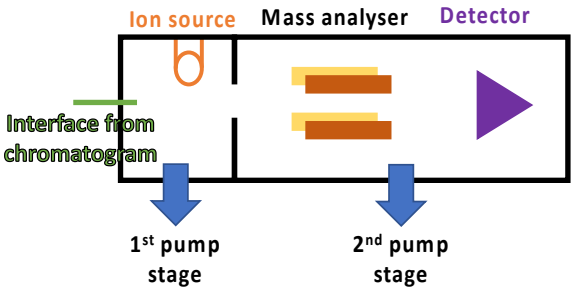

FIGURE 3. Schematic diagram of mass spectrometer (Kellner et al. 2004, 868 Figure 26.1.)

In mass spectrometry, the compounds are ionized either by chemical ionization or electron ionization (EI), of which the latter is the most common technique. Electron ionization is a way to ionize the components entering the mass specrtometer's detector's ion source, in which electrons emitted from a hot filament are accelerated through 70 V before interacting with the sample and ionizing it. Usually the charged electron is most likely to remove the electron with lowest ionization energy. The ion formed from this initial ionization can break into fragments if it has enough extra energy. This causes the molecular peak, which is equal to the compound's nominal mass, to be small or non-existent, as the initial molecule can fragment almost fully. The most intense peak is called the base peak, and all other peaks are expressed as percentages of the base peak intensity. (Kellner et al. 2004, 828, 869; Harris 2010, 503–505.)

Transmission quadrupole mass spectrometer is a common instrument to pair with gas chromatography. The sample passes from the GC column as an ion spray through a heated connector to the EI chamber, which is pumped to maintain a pressure of about 10^{-4} Pa. The vacuum is made using either a high-speed turbomolecular pump or an oil diffusion pump. The quadrupole consists of four metal rods, which are placed parallel to each other. The rods have both constant voltage and radio frequency oscillating voltage applied to them, which in turn allow only ions with particular mass-to-charge ratios to reach the detector. Figure 4 shows a schematic diagram of a quadrupole mass spectrometer. (Kellner et al. 2004, 829–830; Harris 2010, 513–514.)

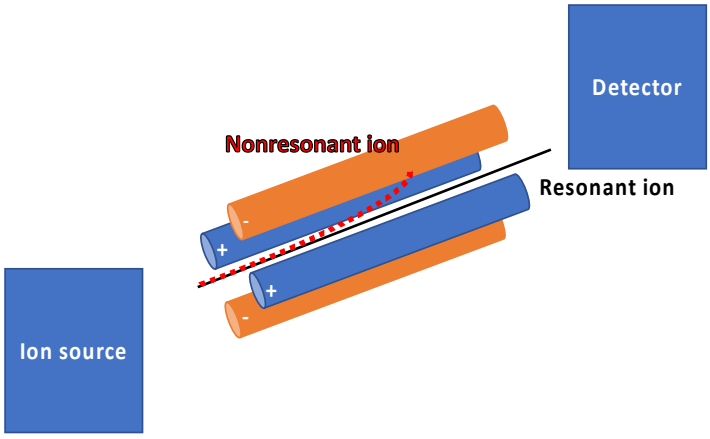

FIGURE 4. Schematic diagram of quadrupole mass spectrometer (Kellner et al. 2004, 830 Figure 25.4.3.)

An electron multiplier is used as a detector in mass spectrometry and its purpose is to amplify the signal and change the energy from ions to electrical current for data collection. Electron multiplier multiplies each ion by emitting a secondary electron when they collide against its walls, thus creating a cascade as each electron and secondary electron emits a new electron upon collision. Electron multiplier has a typical gain of 10^6, and the signal is proportional to the ions coming from the analyser. (Kellner et al. 2004, 831; Jaarinen & Niiranen 2005, 128; Harris 2010, 503.)

4 METHOD DEVELOPMENT AND VALIDATION

4.1 Development

Method development is usually a stage where pre-existing method is tuned for a particular laboratory and their equipment. The method is usually based on a standardized method, equipment manufacturer's application note or a research paper. The document, be it a standard or something else, usually has all the necessary information from sample pre-treatment to calibration mixtures to limit of applications. (Harris 2010, 587–589.)

There is sometimes nothing to base the method on, and in which case a study must be carried out. A useful guide to method selection is to consider the following; the goal of the analysis, what would be most suitable for sample preparation, what detector would work best, what column provides suitable separation, and what injection method should be used. (Harris 2010, 587–589.)

4.2 Validation

Method validation is the process in which an analytical method is proven to be suitable for the intended use. Validation relies heavily on the information collected during method development, interlaboratory studies, or on laboratory specific validation processes, which all generate data about the method's performance parameters. The purpose of validation is to acknowledge the limitations of the method, and how they affect the method, rather than try to correct them all, as that would realistically be impossible. The potentially significant influence factors should therefore be identified, their significance be compared to the overall precision and shown to be negligible. (Ehder 2005, 25; Prichard & Barwick 2007, 73–74; Ellison & Williams 2012, 7.)

Limit of detection (LOD) is the lowest concentration of the analyte that can be detected by the method and which value differs from the blank. Therefore, LOD is located at the lower end of the method's operation range. **Limit of quantification (LOQ)** is the lowest

concentration of the analyte that can be given an error estimate. (Ehder 2005, 29–30; Ellison & Williams 2012, 7; Magnusson & Örnemark 2014, 20–21.)

These values can be calculated for a method or for an instrument. It is important to note that to evaluate the whole method the samples need to go through the whole sample process from the sample preparation to result calculation, as any regular samples would. The instrument detection limit can be evaluated with test samples, spiked blank samples or reagent blanks. (Magnusson & Örnemark 2014, 20-21.)

Both LOD and LOQ are usually calculated by multiplying sample standard deviation (s_0) with a suitable factor. The s_0 should be the precision obtained from typical test samples, and a reliable estimate should be achieved by sufficient replicate measurements. The equations 1 and 2 show respectively the equations for LOD and LOQ concentrations. (Magnusson & Örnemark 2014, 20-21.)

$$c_{\mathrm{LOD}} = \frac{3s_0}{b_1} \qquad (1)$$

where c_{LOD} is the term used for LOD, s_0 is sample standard deviation and b_1 is the slope of the calibration curve, and the factor is 3.

$$c_{\mathrm{LOQ}} = \frac{10s_0}{b_1} \qquad (2)$$

where c_{LOQ} is the term used for LOQ, s_0 is sample standard deviation and b_1 is the slope of the calibration curve, and the factor is 10. (Ehder 2005, 30; Harris 2010, 103.)

5 METHODOLOGY

5.1 Instrumentation and settings

The practical portion of the work was conducted with an Agilent GC-MS system, and its technical details can be found in table 3. The system had 8 sample turrets. The program connected to the system was Agilent's MS ChemStation, but the results were analysed with Agilent's MassHunter Qualitative program. The final method and its details can be found in appendix 2.

TABLE 3. Instrumentation

GC	Agilent Technologies
	6890N Network GC system
MS	Agilent Technologies
	5973 Network Mass Selective Detector
Injector	Agilent Technologies
	7683 Series Injector
	8 sample turrets
Pump	Turbo pump
Programs	MS ChemStation
	MassHunter Qualitative
Column	DB-5MS

5.2 Calibration and Standard Solutions

The reagents used for calibration and their supplier information can be found in table 4. The PAH standard mix came in 1 ml glass vial. The reagents for ISTDs were solid and thus the solutions for them were done by hand in the laboratory.

TABLE 4. Reagents and their supplier information

Reagent	Supplier	Order no.	Used for/as
PAH standard mix	Merck (Sigma-Aldrich)	CRM47940	Certified reference material (TraceCERT); 10 µg/ml in acetonitrile
Chrysene-d12	Merck (Sigma-Aldrich)	442523	ISTD
Acenaphtene-d10	Merck (Sigma-Aldrich)	442432	ISTD
Phenantrene-d10	Merck (Sigma-Aldrich)	442753	ISTD

Chrysene-d12 was left out of the combined PAH ISTD due to unsatisfactory dissolving into the solvent. N-hexane was used as solvent as it was the final solvent used in the extraction process. The dilution sequence of internal standard solutions is shown in table 5.

TABLE 5. ISTD dilution sequence

	m mg	V ml	c mg/ml	L1 µg/ml	L2 µg/ml	L3 µg/ml
Chrysene-d12	9.9	50	not in solution as did not dissolve completely			
Acenaphrene-d10	10.15	50	0.203	10.15	2.03	0.0406
Phenantrene-d10	10.05	50	0.201	10.05	2.01	0.0402

The L1 dilution was made from separate base solutions, which concentrations and volumes are shown in table 6. The L1 was diluted in 5 ml : 100 ml, the L2 in 1 ml : 5 ml from L1, and L3 was to be added to the acetone used in extraction in ratio 1 ml : 50 ml.

5.3 Sample preparation

The validation did not extend to sample preparation, and it is therefore presented in its basics, and as the validation continues and the method sees more use, the practices described in this section may change slightly.

The sample matrixes for this method were ash, soil and sand. Before validation the sample preparation was tested with all three. The ash used in this test was from a private homeowner's fireplace, the soil used was commercially sold soil for plants, and the sand was from a domestic sandbox. The tests were conducted for choosing a sample preparation process out of two possible solutions, one of which was based on Nordtest report 329, and the other was a combination of the standards SFS-EN 15527:2008 and SFS-ISO 18287:2006. The sample preparation processes are described shortly in table 6. It is to be noted that all glassware used had PTFE tops.

TABLE 6. Tested sample preparation methods. Method A is based on standards SFS-EN 15527:2008 and SFS-ISO 18287:2006, and method B is based on Nordtest report 329

Method A	**Method B**
- Weight approximately 20 g of sample - Add 50 ml of acetone containing ISTD - Extraction on shaker machine for 30 min - Add 50 ml of n-hexane - Repeat extraction for 30 min - Allow to settle - Decant the supernatant - Wash the remaining solids with 50 ml of n-hexane - Decant the supernatant - Transfer the sample to separating funnel and add 400 ml of water to wash the acetone out - Take sample I from the solvent phase - Separate the water from the sample - Dry with Na_2SO_4 - Wash with 10 ml of n-hexane three (3) times - Take sample II	- Weight approximately 20 g of sample - Add 50 ml of acetone containing ISTD - Add 50 ml of n-hexane - Extract for 60 min on shaking machine - Add 400 ml of water - Shake well - Take sample I from the solvent phase - Dry with Na_2SO_4 - Shake well - Take sample II

As can be seen from the table 6, the method B produces less waste than method A, and it has less steps than method A, which makes it less labour intensive, and as there were no major differences with the chromatograms between the methods, it was decided that method B was to be used for further tests.

6 RESULTS

This section covers the validation results of the method. The formulas used in the validation are presented in section 4.2 Validation. Calibration curves can be seen in appendix 3.

The integration of the peaks was done with MassHunter Qualitative program, using integrate MS/MS function, which integrated by peak area of relative area larger or equal to 1.000 % of the largest peak, with hand integrating when needed, and the calculations were done in Microsoft Excel 2013 with information analysis regression- tool. The validation consisted of instrument detection and quantification limits.

LOD and LOQ were calculated with their respective equations 1 and 2, which are presented in section 4.2 Validation. Equations 3 and 4 show the calculations in case of naphthalene, and table 7 shows the limits of all compounds.

$$\begin{aligned} c_{\mathrm{LOD}}(\text{naphtalene}) &= \frac{3 \cdot 34805}{92291}\ \frac{\text{mg}}{\text{ml}} \\ &= 1{,}13\ \frac{\text{mg}}{\text{ml}} \\ &\approx 2\ \frac{\text{mg}}{\text{ml}} \end{aligned} \tag{3}$$

$$\begin{aligned} c_{\mathrm{LOQ}}(\text{naphtalene}) &= \frac{10 \cdot 34805}{92291}\ \frac{\text{mg}}{\text{ml}} \\ &= 3{,}77\ \frac{\text{mg}}{\text{ml}} \\ &\approx 4\ \frac{\text{mg}}{\text{ml}} \end{aligned} \tag{4}$$

The limits are calculated from a calibration curve run, but some compounds did not have enough data points from said calibration curve for regression analysis, in which case all available data was used in the regression analysis. All the values are rounded up to the next even number, to maintain reliable accuracy.

TABLE 7. Detection and quantification limits

	Calibration curve		**All data**	
	LOD µg/ml	**LOQ µg/ml**	**LOD µg/ml**	**LOQ µg/ml**
Naphthalene	2	4	0.4	1.2
Acenaphtene	2	6	0.4	1.2
Acenaphtylene	1	2	0.4	1.1
Fluorene	2	5	0.3	1.0
Anthracene	2	6	0.3	1.0
Phenanthrene	2	5	0.4	1.2
Fluoranthene	2	6	0.3	0.9
Pyrene	2	5	0.3	0.8
Benz[a]anthracene	3	10	0.7	2.1
Chrysene	4	12	0.6	2.0
Benzo[b]fluoranthene	3	10	1.5	5.0
Benzo[k]fluoranthene	3	8	1.3	4.2
Benzo[a]pyrene	2	6	1.4	4.7
Indeno[1,2,3-cd]pyrene *	–	–	2.8	9.1
Dibenz[a,h]anthracene *	–	–	3.0	10.0
Benzo[ghi]perylene	1	4	2.0	6.6

* Calculated only from all data as only two data points could be identified from the calibration curve samples

It is to be noted, that these limits are instrument limits, not method limits and method limits are usually higher than instrument limits. The implications of this and the results shown in table are discussed in the next section.

7 DISCUSSION

The aim (provide the laboratory with an instrumental method capable of detecting PAHs) and objective (to develop the method and validate it for use of the laboratory) were met during this study. The validation needs more work but is easy to continue as the sample preparation and instrumental method are finalised by this study.

The problems that affected this work were as follows: too few vial places in the autosampler tower (ASL) and the time limitations with the laboratory. The first problem was perhaps the direst, as the ASL only had eight turrets and combined that to the length of the method (35 minutes) and the fact that the laboratory only operates eight hours per weekday, makes diverse overnight runs practically impossible, as changing sample vials during the run is risky at best, and could ruin the run and the sampler at worst.

Other problems that need addressing are the scarcity of small glassware and bottle tops made out of PTFE or glass, as the solutes are very volatile and therefore the analysis suffers in terms of precision if the bottle tops are not out of suitable material, and as the calibration standards are of small volume, a bigger glassware is not feasible.

Waste management and the cleanliness of the glassware are another concern and should have clear rules that are easy to follow. There are times in the analysis procedure when it is necessary to use disposable glass Pasteur pipettes, which contaminates them in PAHs. The problem goes as following: should one use each pipette the one time only to prevent contamination or should the pipettes be washed. In the second case, there is the potential problem of cross contamination between the compounds if the washing is insufficient. The first problem is the disposal if used only once, as the compounds are classified as persistent organic pollutants and need specific disposal. If they are disposed are they disposed to the sharp object recycling bin or should contaminated things have their own recycling bin? The contamination is an issue with all the glassware, but the acetone wash or deactivation with 20 % nitric acid should help the matter. (Sirén, Perämäki & Laiho 2009, 194.)

As always during washing the glassware one should also consider where the wastewater goes and what are the possible problems with the wastewater. For example, the

wastewater could end up in nature if it is not treated accordingly before it makes its way to water treatment plant, as water treatment plants are primarily for residential purposes and might not have the means to take care of the contaminants accordingly. This is a problem with target compound, as PAHs are highly toxic to aquatic life in the presence of UV light (sunlight) (Walker 2001, 171).

As seen from table 7 in section 6, the detection and quantification limits are not within the recommended limits of application presented in standards SFS-EN 15527:2008 (0.01 mg/kg) and SFS-ISO 18287:2006 (0.1 mg/kg), which indicates the need for further validation. It is to be noted that the validation was implemented in a much smaller scale than what was first planned. Especially sample preparation needs more data to be fully comprehensive regarding the internal standard, and all the calibration solutions need to be made with constant internal standard addition, which hasn't been possible with the available reagents or equipment. It is also advisable to do cross laboratory comparison with previously analysed samples, to compare the method with other methods made for the same purpose.

The sample preparation and calibration solutions need more work to be efficient enough for the laboratory's needs. New calibration points should be considered as some compounds could not be validated from the calibration curve, and there should be more than five points, so that the occasional outlier could not ruin the whole batch. The data from the validation also supports this need. Mostly the calibration and internal standard solutions need to have more practical dilution processes, and degradation studies should be made, and best conditions to prevent degradation need to be implemented. The process of result calculations needs to be refined before this method is implemented more widely, as learning how to use new software takes time and training to learn how to use effortlessly. It has also been made clear, that the method should also to be carried over and validated to another apparatus for more efficiency in the laboratory.

A faulty electron multiplier could also be one of the reason for the insufficient data from the validation, as the response factor is usually 10^6, and the factor was considerably lower on some results. Another reason for such a low response factor might be the degradation of the calibration solutions. The validation and result calculation would also have benefitted from Excel having options for the curve weighting. The MassHunter software

does offer this option and it is worth considering using it for the whole of the calculations in the future.

In the future, there needs to be a definite protocol for the samples – what dilutions and/or standard additions are most likely to fall within the range and are all samples prepared at the same time, or should the dilution or addition be done after the results have come from the first sample. Protocol will make the analysis more proficient, which is something every laboratory wants.

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APPENDICES

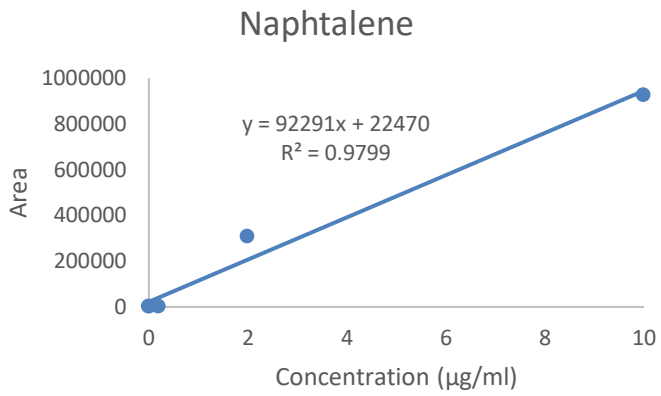
Appendix 1. Instrumentation

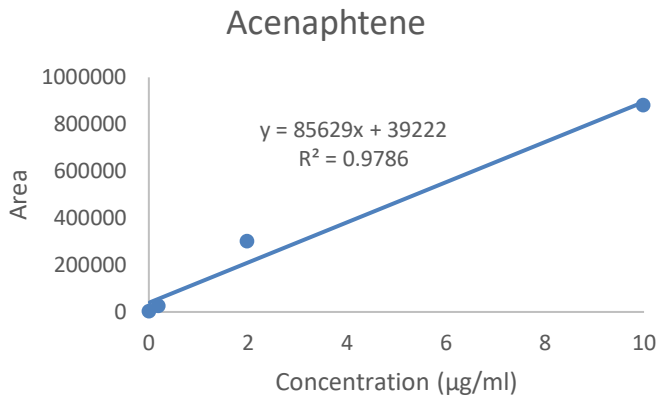
Injector			Oven		
Volume	1.0 µl × 1 = 1 µl		Setpoint	75 °C	
	Pre	Post	Equilibrium time	0.5 min	
Sample washes	3		Max temperature	325 °C	
Solvent A	3	3	Rate	Temperature	Hold
Solvent B	3	3	°C/min	°C	min
Sample pumps	6			75	1
Inlet			25	250	0
Heater	300 °C		3	310	7
Pressure	19.3 psi		Total time		35
Total flow (He)	35.4 ml/min		Solvent delay	4 min	
Splitless	20.6 ml/min	1 min			
Gas saver 20.0 ml/min after 2 min			EMV mode gain factor		
Column			Gain factor	5.00 = 1106 V	
Column	DB-5ms		Acquisition mode	SIM	
Constant flow			Real time plot		
Pressure	19.3 psi		Time window	60 min	
Flow	1.0 ml/min		MS Window 1	Plot type total	
Average velocity	40 cm/sec		Y scale 0 to 2000 000		

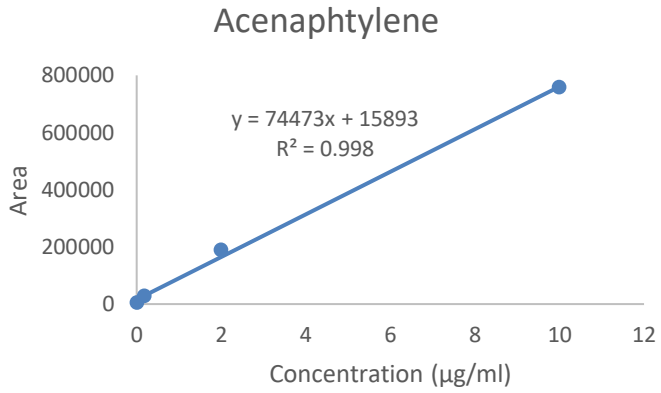
PAH	start time	1. peak	2. peak	3. peak	4. peak	5. peak
Naphtalene	4.00	128.1	102.1	74.0		
Acenaphtene	6.45	152.1	76.1	63.1		
Acenaphtylene	6.60	154.1	76.1	63.1		
Fluorene	7.15	166.1	82.3	122.0		
Anthracene	8.12	178.1	152.1	75.9		
Phenantrene	8.20	178.1	152.1	89.1		
Fluoranthene	9.65	202.1	101.1	88.1		
Pyrene	10.05	202.1	101.1	174.1		
Benz[a]anthracene	12.95	228.2	114.1	100.9		
Chrysene	13.15	228.2	112.9	100.9		
Benzo[b]fluoranthene	16.90	252.2	126.1	112.8		
Benzo[k]fluoranthene	17.04	252.2	126.1	112.9		
Benzo[a]pyrene	18.20	252.2	124.9	112.9		
Indeno[1,2,3-cd]pyrene	23.10	276.1	207.0	138.1	278.1	139.0
Dibenz[a,h]anhtracene	23.43	278.1	207.0	139.0	276.1	138.1
Benzo[ghi]perylene	24.25	276.1	207.0	138.1		

Appendix 2. Calibration curves

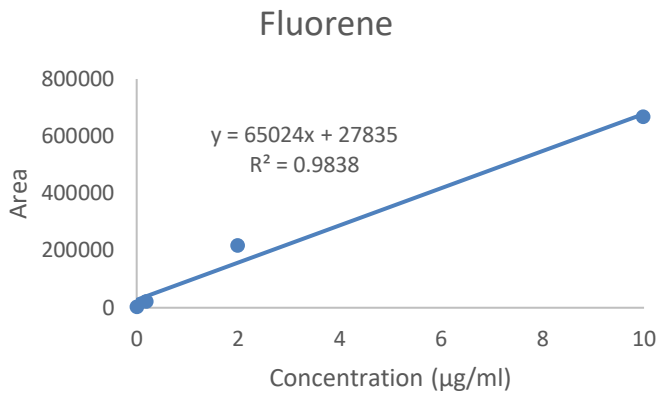
1 (4)







(cont.)


Fluorene
800000
600000
400000
200000
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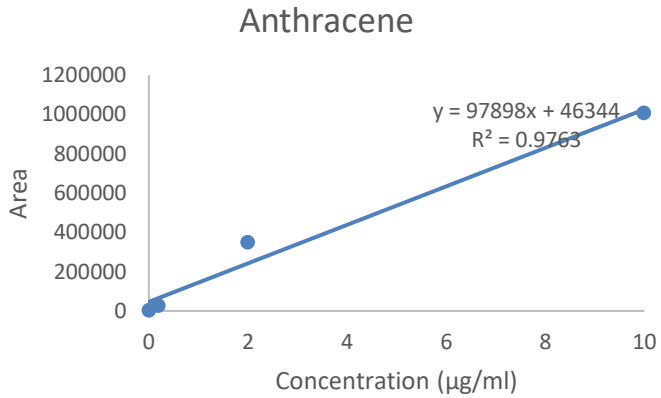
R² = 0.9838
0
2
4
6
8
10
Concentration (µg/ml)


Anthracene
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800000
600000
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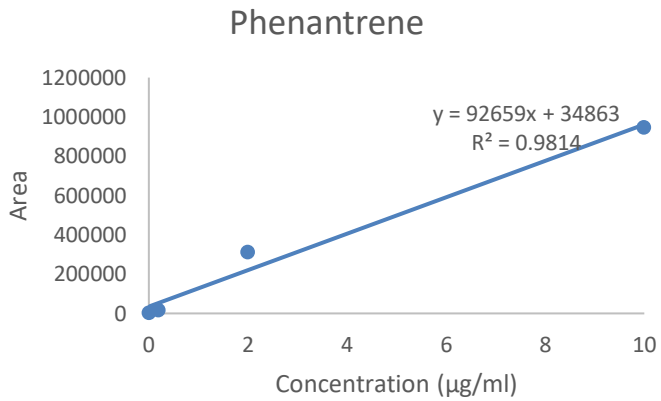
4
6
8
10
Concentration (µg/ml)


Phenantrene
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800000
600000
400000
200000
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R² = 0.9814
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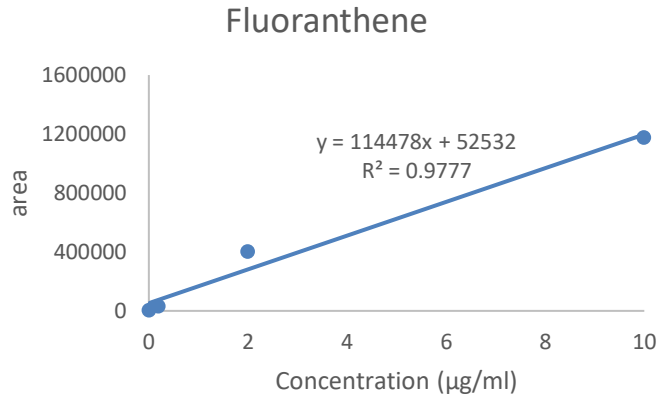
2
4
6
8
10
Concentration (µg/ml)


Fluoranthene
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1200000
800000
400000
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R² = 0.9777
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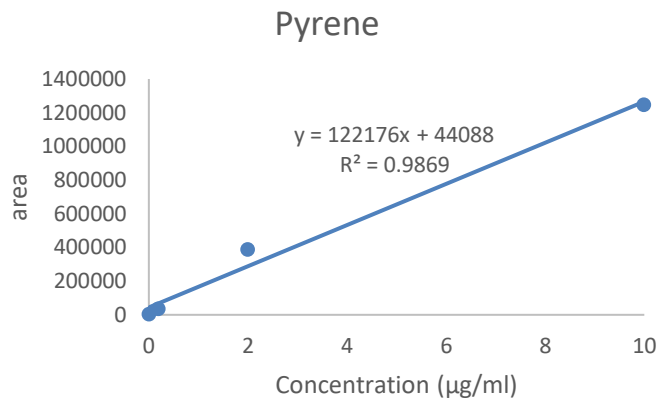
2
4
6
8
10
Concentration (µg/ml)

3 (4)


Pyrene
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R² = 0.9869
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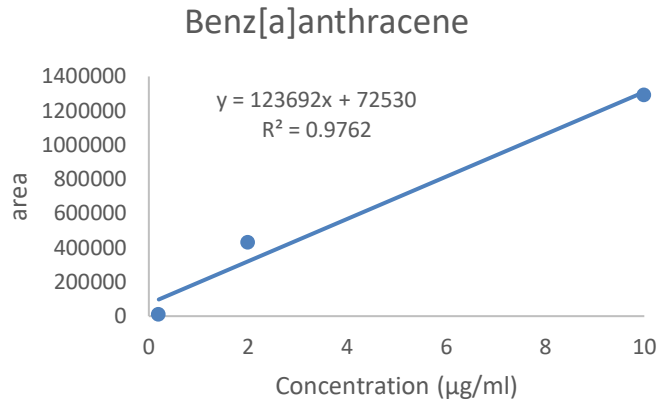
1000000
800000
600000
400000
200000
0
0
2
4
6
8
10
Concentration (µg/ml)


Benz[a]anthracene
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R² = 0.9762
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800000
600000
400000
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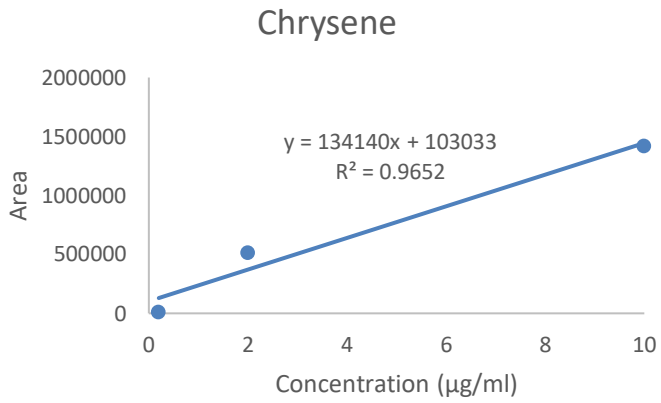
0
2
4
6
8
10
Concentration (µg/ml)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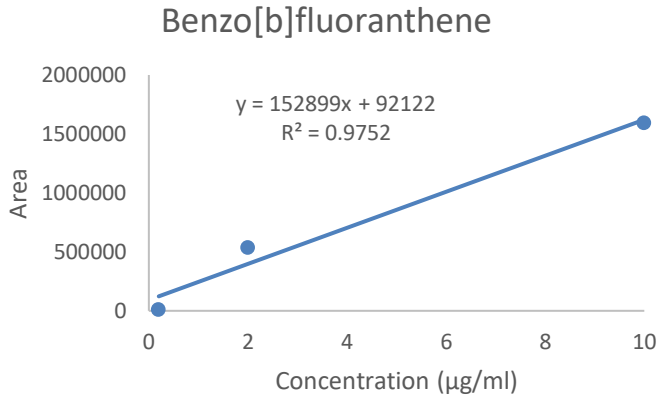
4
6
8
10
Concentration (µg/ml)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R² = 0.9752
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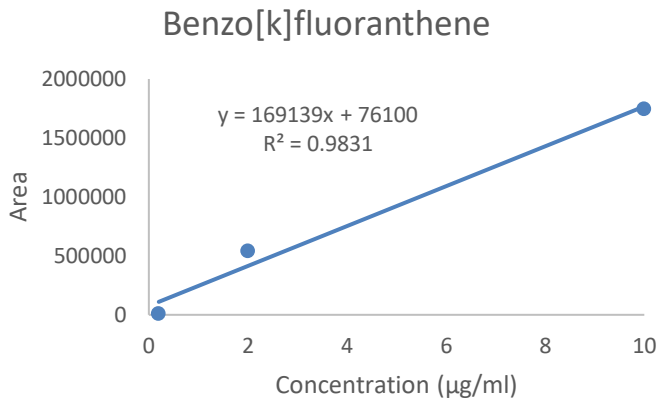
500000
0
0
2
4
6
8
10
Concentration (µg/ml)

4 (4)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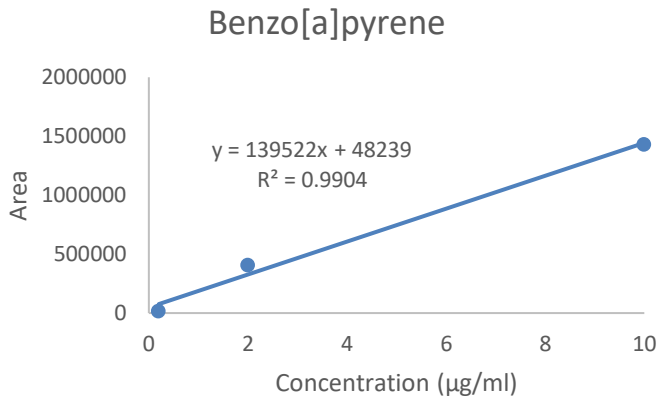
2
4
6
8
10
Concentration (µg/ml)


Benzo[a]pyrene
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R² = 0.9904
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1500000
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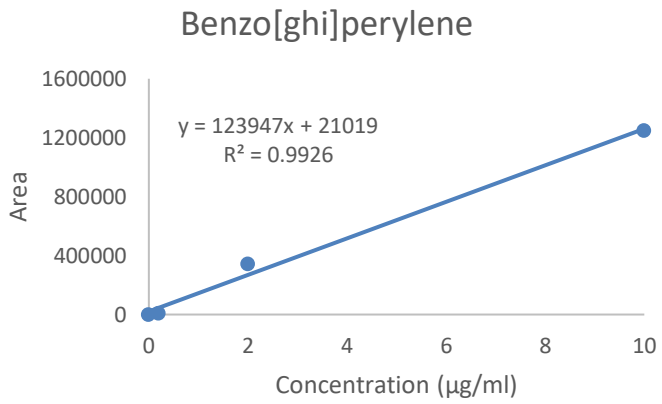
0
2
4
6
8
10
Concentration (µg/ml)


Benzo[ghi]perylene
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R² = 0.9926
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1600000
1200000
800000
400000
0
0
2
4
6
8
10
Concentration (µg/ml)