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CHEMICAL ANALYSIS IN CONTAMINATED SOIL

RESEARCH ARTICLE

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Application of the concept of design space in method development for the determination of contaminants in soil

Jelena Jurjeva[a] and Mihkel Koel[b]

[a] The J. Molner Company OÜ, Akadeemia tee 21–5, 12618 Tallinn, Estonia

[b] Department of Energy Technology, Tallinn University of Technology, Akadeemia tee 15, 12618 Tallinn, Estonia

ABSTRACT

The analysis of polycyclic aromatic hydrocarbons and polychlorinated biphenyls is an important topic in environmental analysis, and several analysis procedures have already been proposed. Following the approach of analytical quality by design (AQbD) contributes to the development of simultaneous identification and quantification of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), alkylated PAHs and aliphatic compounds in soil by the gas chromatography-mass spectrometry (GC-MS) analytical technique, using the mechanical extraction method for sample preparation, which results in the modified method with superior analytical parameters. In the study, the set-up of design space (DS) for method development was introduced, including the selection of an appropriate gas chromatography (GC) column. Subsequently, design of experiment enabled finding the optimal GC conditions for the new method that allowed achieving adequate resolution and recovery. Method development involved changes in sample preparation, making it simpler and faster. Decreasing the number of different extraction steps enabled reducing staff numbers and consumable costs. As a result, methods of analysis of PAHs, PCBs and aliphatic and aromatic compounds in a single run were developed, offering reliable accuracy, precision, linearity and recovery rates that fully comply with Swedish regulations. This new method and its introduction to laboratory practice was validated, and the greenness of the method was evaluated.

Keywords:
environmental chemistry, method validation, gas chromatography, experimental design, analytical quality by design, design space

Corresponding authors:
Jelena Jurjeva
jurjeva.jelena@gmail.com
Mihkel Koel
mihkel.koel@taltech.ee

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1. Introduction

Analytical quality by design (AQbD) – the term mostly used in the pharmaceutical industry – aims to integrate analysis and chemical state control development, resulting in more reliable analytical procedures that need less effort for performance verification and post-approval changes because of a better-defined design space (DS) – the region where all the input parameters can vary without altering the quality of the product [1].

According to the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use [2], the setting of this space starts from the specifications of a product or the quality target product profile (QTPP), which forms the basis for the design and development of the product. It summarizes all the terms of processes, product properties, product quality criteria as well as safety issues both for the production process and the product itself through the use of which the product can have the desired quality. The next step is directly related to the identification of critical quality attributes (CQAs) of the product, defining the appropriate limits for the product properties or distribution to ensure the desired product quality [2]. Here the direct relationship with quality risk management comes into play to identify which material attributes and process parameters impact CQAs [2,3]. This approach was originally designed for the production process, but similar principles can be applied to the environmental analysis of soil, water or air, where the quality of analysis is an important characteristic describing the state of the environment and controlling the quality of remediation.

DS is important in relation to quality management because working within DS is not considered a change. DS allows to react when the ‘process drifts’ are observed that could be caused, for example, by changes in analysed materials, such as different types of soil or water that cannot be actively controlled in order to keep the quality of environmental parameters at the intended level. Such changes within an approved

DS do not require the authorities' notification or preapproval [4], while the movement out of DS does and should normally initiate a post-approval regulatory change process [5].

1.1. Design space in the development of analytical procedures

There are different approaches and methods for defining DS and its relationship with the product – in the present case, the environmental parameters [6]. There are no official guidelines for establishing these kinds of parameters, and different methods can be used for this purpose, for example, literature search and relevant statistics [7].

Developers of analytical measurements are often asked the following questions: what methods are available for the measurements? What are the characteristics of the samples? What analytical performance criteria are requested by regulatory agencies? What is the method-provided performance? What laboratory resources are available? These questions and the related answers enable the developers to define the exact concept of the method and the limits for analytical procedures, which in principle form the DS for a particular analytical process.

The environmental quality and level of monitoring define and constitute the basis for the control and limits of the environmental parameters in DS, and the procedure development and control are aligned with a similar DS for the analytical parameters. The analytical target profile (ATP) is similar to the environmental parameters profile, which prospectively summarizes the requirements associated with the quality attributes of the measurements that need to be met by an analytical procedure. The general criteria defined in ATP relate to the maximum uncertainty associated with the reportable result that gives an acceptable confidence in the quality of the decision. Other important performance characteristics for each ATP are specificity, accuracy and precision, taking into account the expected range of the analyte [8]. One must keep in mind that ATP does not refer to a specific analytical technique or an operative mode; rather it establishes the criteria required for the technique as well as the measurement parameters. During the method search phase, prior knowledge is gathered, and, if necessary, preliminary experiments are carried out. In separation procedures, which are common in environmental analysis, stationary phases and solvents in chromatography, operative mode/pseudo-stationary phases in liquid chromatography as well as sample preparation are quickly evaluated or selected based on the literature, univariate experiments and/or the laboratory's experience, with the aim of approaching ATP. Regarding sample preparation, the choice of target concentration values as well as the preparation of reference solutions should be considered, paying attention to high performance and efficiency. For example, in environmental sample preparation, one needs to carefully consider the selection of analyte(s), pay due attention to the properties of the extraction solvent as well as carry out the sampling of the environmental probe in a representative way.

In environmental analysis, the setting of an ATP depends on the threshold values of contaminants established by relevant authorities for each case (air, water, soil). Compliance control requires using reliable and reproducible methods for sampling, sample pre-treatment prior to analysis and analytical measurements to produce results that are valid for legal purposes. This stresses the importance of the methods of validation, which is especially relevant in the development of reliable analytical procedures [9,10]. Quality parameters such as accuracy and robustness are therefore evaluated only at the end of the method development process. Notably, method robustness, defined by the ICH guideline Q2(R1) as 'a measure of the capacity of the method to remain unaffected by small but deliberate variations in method parameters' [11], is a key point in assessing the quality of an analytical procedure.

AQbD involves good understanding and control of an analytical method, based on quality risk management [12]. Risk assessment can be carried out using several tools. In the first place, for visualisation it is helpful to use a fishbone or Ishikawa diagram, which identifies potential factors and distributes factor-related risks into categories associated with instrumentation, materials, methods, measurements, laboratory environment and human factors [13]. In addition to the above, the recognition of significant factors for DS and their optimization can be made using statistical analysis and methods of design of experiment (DoE) [14].

In this study, the concept of DS was applied to developing a modified analytical method for compounds from oil-spills, namely polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and aliphatic and aromatic fractions, which are known as accumulating contaminants. PAHs are formed mainly during the incomplete combustion of organic materials. PCBs can be released into the soil by leakages from landfills with PCB-containing products and disposed industrial waste [9]. ATP was related to the development of a precise and accurate method for determining oil compounds in soil and sediment using the gas chromatograph-mass spectrometry (GC-MS) technique in order to support uncertainty estimation throughout the whole range of analyte concentrations and achieve the necessary limits of quantification (LoQ). In this case, the peak areas on chromatograms and the resolution between the peaks were used as critical methods attributes (CMAs).

An analytical target in this case is the oil spill into the soil and the compounds that are spilled, namely 16 parent PAHs and their alkylated analogues, seven PCBs, four aliphatic fractions from octane to pentatriacontane and different aromatic compounds. The concept of DS applied to the target compounds in this study consists of the following steps: identification of critical method parameters, performance assessment, establishment of acceptable ranges, optimization of conditions, method validation, monitoring and control.

1.2. Legal limits of design space for oil products in the environment (Scandinavian examples)

An increasing number of countries have established threshold values to monitor and evaluate the content of contaminants in soil, water and air. Compliance control requires using reliable, reproducible and validated methods for sampling, sample pre-treatment prior to analysis and analytical measurements to produce results valid for legal purposes [10]. This way, DS is referring to a multidimensional combination of method parameters and conditions that ensures method robustness, reliability and performance [15]. The use of chemometrics in environmental studies is a powerful tool that distinguishes three main areas of interest: quantitative chemical analysis, monitoring for environmental quality assessment and prediction of toxicological effects [16]. It is particularly important in the development of analytical methods for complex analyses, such as the determination of PAHs, PCBs and aliphatic and aromatic compounds in a single run.

The compounds used for this study are emerging contaminants, which were chosen due to their potential risks and increasing occurrence in soil and sediment. In Scandinavian countries, great attention is paid to the pollution of soil and its remediation [17]. In Norway, a set of guidelines has been developed by the Norwegian Environment Agency [18,19]. The Environmental Damage Fund, managed by the Ministry of the Environment, is responsible for compensating for the costs of remediating oil-contaminated soil and groundwater when the party that caused the accident is not known [20]. The Soil Contamination Act, which entered into force on 1 January 2000 in Denmark, has two main objectives: one is groundwater protection and the other is prevention of health problems that may arise from the contaminated areas [21,22]. The Swedish Environmental Code harmonizes the general rules and principles in environmental legislation [23]. The Swedish Environmental Protection Agency has established guidelines and standards for acceptable levels of contaminants in soil (Table 1) [24,25].

Several methods exist in Sweden for analysing oil compounds in soil. First, there is a method devised by the Swedish Environmental Protection Agency, which was then further developed under the so-called SPIMFAB project (Svenska Petroleum Institutet Miljösaneringsfond AB) [26] for analysing aliphatic fractions in soil. Also, the instruction for carrying out the analysis of the aromatic fractions >C16–C35 [27] is available. In addition, a method for analysing PAHs that meets regulatory requirements of contaminants in soil can be used. Table 1 presents the limits of contaminants in the living and industrial zones for PCB-7 (sum of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180), PAH-L (sum of light PAHs: naphthalene, acenaphthene, acenaphthylene), PAH-M (sum of medium PAHs: fluorene, phenanthrene, anthracene, fluoranthene, pyrene), PAH-H (sum of heavy PAHs: benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene) as well as for different aromatic and aliphatic fractions. Table 2 lists the limits of contaminants in the living zone for aliphatic fractions and PAHs in Denmark. In that country, all the methods of analysis developed must comply with the existing soil quality criteria for oil and/or petrol products in soil and must be carried out according to the methods prescribed by the Danish Environmental Protection Agency [22].

Table 1. Concentration limits for contaminated land in Sweden [25]

Compound	Limit in the living zone, mg kg^{-1}	Limit in the industrial zone, mg kg^{-1}
PCB-7	0.008	0.2
PAH-L	3	15
PAH-M	3	20
PAH-H	1	10
Aliphatic > C8–C10	20	120
Aliphatic > C10–C12	100	500
Aliphatic > C12–C16	100	500
Aliphatic > C16–C35	100	1000
Aromatic > C10–C16	10	50
Aromatic > C10–C16	3	15
Aromatic > C16–C35	10	30

Table 2. Soil quality criteria given by the Danish Environmental Protection Agency for sensitive lands [21,22,28]

Compound	Soil quality criteria for sensitive land, mg kg^{-1}
C6–C10	25
> C10–C15	35
> C15–C20	55
> C20–C40	150
C6–C40	150
PAHs, total*	1.5
Benzo(a)pyrene	0.1
Dibenz(a,h)anthracene	0.1
PCBs, total	0.02

* defined as the sum of individual components: fluoranthene, benzo(b,k)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene and indeno(1,2,3-cd)pyrene

The values for the hydrocarbon fractions given in the Danish regulation differ from those presented in the Swedish regulation. The difference may be mainly due to the fact that the hydrocarbon values in Denmark were determined by gas chromatography flame ionization detector (GC-FID) and in Sweden, by GC-MS. For the PAHs analysis, both countries used GC-MS.

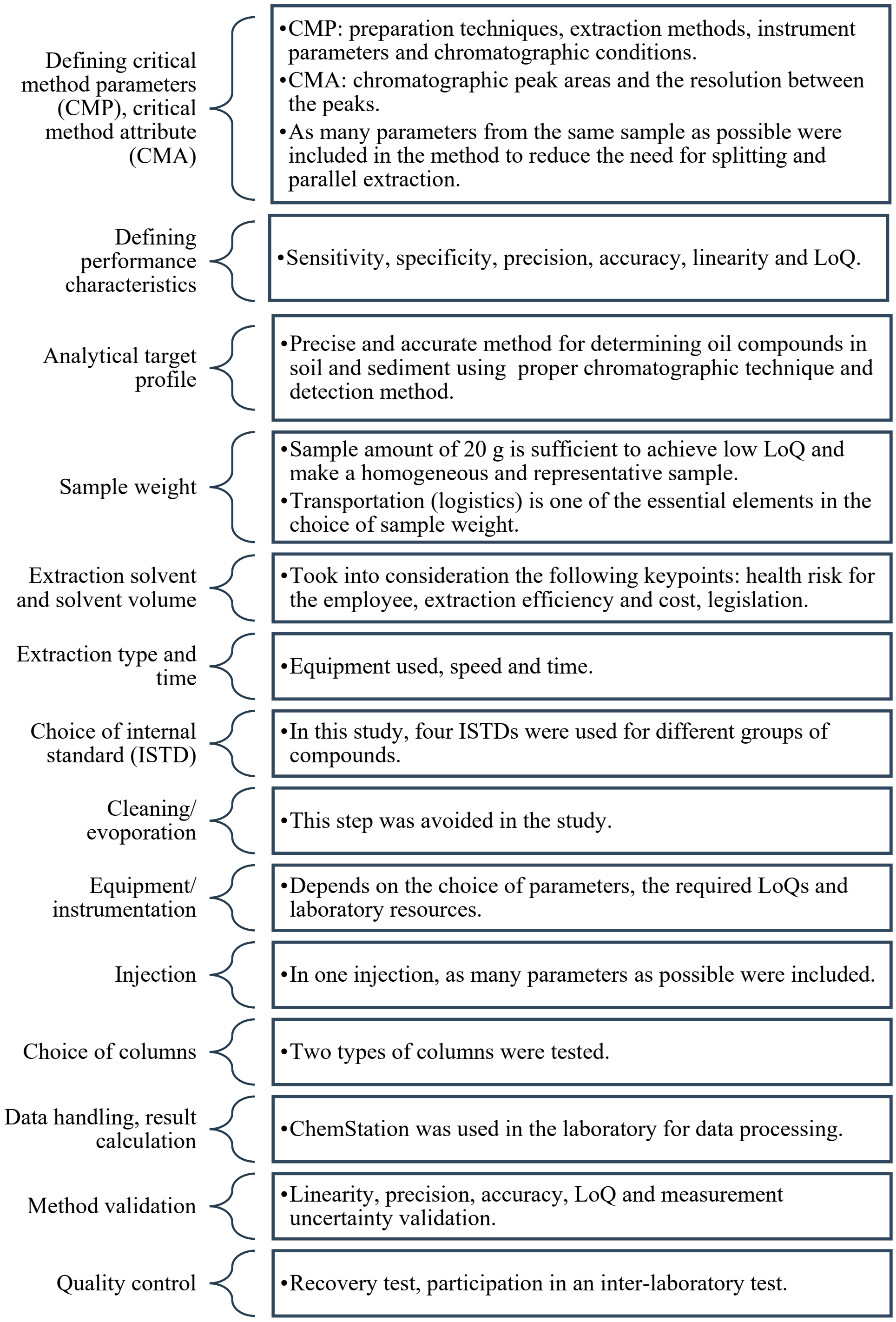
2. Steps for the new method development

2.1. Analysis scheme

In this study, the legal aspect of DS for oil compounds in soil is based on the guidelines devised by the Swedish Environmental Protection Agency. Also, the analytical performance criteria requested were defined by this agency. In soil analysis, the number of the samples to be analysed is usually relatively high, and the analysis method has to be applicable to different types of soil.

In the present study, all samples were analysed for aliphatic and aromatic fractions as well as for PAHs, and approximately 80% of samples for PCBs. It means that the combined method was highly preferable for analysing all compounds in one run.

The concentration limits of contaminants set in regulations and the expected detection limits require defining the critical method parameters for method development. The flow chart (Fig. 1) shows the steps of the concept of DS applied to the target compounds in this study.



Defining critical method parameters (CMP), critical method attribute (CMA)	•CMP: preparation techniques, extraction methods, instrument parameters and chromatographic conditions. •CMA: chromatographic peak areas and the resolution between the peaks. •As many parameters from the same sample as possible were included in the method to reduce the need for splitting and parallel extraction.
Defining performance characteristics	•Sensitivity, specificity, precision, accuracy, linearity and LoQ.
Analytical target profile	•Precise and accurate method for determining oil compounds in soil and sediment using proper chromatographic technique and detection method.
Sample weight	•Sample amount of 20 g is sufficient to achieve low LoQ and make a homogeneous and representative sample. •Transportation (logistics) is one of the essential elements in the choice of sample weight.
Extraction solvent and solvent volume	•Took into consideration the following keypoints: health risk for the employee, extraction efficiency and cost, legislation.
Extraction type and time	•Equipment used, speed and time.
Choice of internal standard (ISTD)	•In this study, four ISTDs were used for different groups of compounds.
Cleaning/ evoporation	•This step was avoided in the study.
Equipment/ instrumentation	•Depends on the choice of parameters, the required LoQs and laboratory resources.
Injection	•In one injection, as many parameters as possible were included.
Choice of columns	•Two types of columns were tested.
Data handling, result calculation	•ChemStation was used in the laboratory for data processing.
Method validation	•Linearity, precision, accuracy, LoQ and measurement uncertainty validation.
Quality control	•Recovery test, participation in an inter-laboratory test.

Fig. 1. Setting DS for oil products in soil.

2.2. Critical method parameters

There are several critical method parameters to choose from, such as the conditions of extraction in sample preparation, GC-MS instrument parameters and the chromatographic conditions in the analysis of contaminants. All these conditions and parameters have a significant impact on the performance of the analysis. The next step is to assess the influence of each critical method parameter on the performance characteristics, such as sensitivity, selectivity, precision, accuracy, linearity and LoQ. For that, the method development experiments and, respectively, the results were evaluated to establish the acceptable criteria for each critical parameter. The further step was to optimize the analytical procedure conditions to achieve the best compromise between sensitivity, selectivity and other performance criteria. Here the peak areas on chromatograms and the resolution between the peaks were CMAs because these are directly related to the strict threshold values of the content of contaminants in the soil established by the authorities [25]. After this kind of optimization, the method was validated using standard procedures. To ensure that the method remains robust and reliable over time, the monitoring and control of critical parameters was performed by a recovery test and participation in an interlaboratory test.

3. Method optimization

3.1. Preliminary studies

The GC-MS technique was chosen for determining oil compounds in soil and sediment because of its ability to separate compounds in complex mixtures and support accuracy and precision within the whole range of analyte concentrations required by official regulations [25,26].

The method for the PAHs analysis was based on the former laboratory method [29] with GC-MS instrumentation used. The analyses of the target compounds were performed on an Agilent 7890B GC system that was equipped with a mass-selective detector 5977B MS. Experiments were conducted using different soil types (loamy, clayey, sandy), different weights, extraction volumes, time and speed as well as two types of GC columns (Rxi-XLB (low-polarity proprietary phase) and Rxi-17Sil (midpolarity crossbond phase)) to evaluate the impact of the above parameters on performance characteristics (sensitivity, selectivity, precision, accuracy, linearity and LoQ). Both columns, according to the recommendation of the producer (RESTEK), exhibit excellent inertness and selectivity for active environmental compounds, such as PCB congeners and PAHs. However, for aliphatic compounds, no information about selectivity was available. Preliminary experiments were conducted using the chromatographic conditions recommended by the column producer for the PAH and PCB analyses. A mixture of target compounds diluted in n-hexane was injected into GC-MS. The standards used were: calibration/window defining hydrocarbon standard (C8–C40 1000 mg/L of each compound) from AccuStandard, New Haven, USA; custom mix, multistandard alkylated PAHs and aromatics in toluene (100 mg/L of each compound) from Chiron, Trondheim, Norway; PAH mix-9 100 ng/μL in acetonitrile from Dr. Ehrenstorfer GmbH, Augsburg, Germany; PCB mix-3 (10 ng/μL in iso-octane) from Dr. Ehrenstorfer GmbH. Both columns showed sufficient resolution for PAHs and PCBs; however, Rxi-XLB column was selected as the analytical column because it produced the best peak shape for aliphatic compounds.

An initial screening study of the analytical procedure was carried out examining the oven and inlet temperatures, flow rate and temperature gradient – the parameters known to have a significant impact on peak retention and separation in GC-MS analysis. The LoQ, reproducibility and measurement uncertainty experiments were conducted and calculated according to the Nordtest guide [30]. Table 3 shows results from the preliminary study.

3.2. Selection of chromatographic parameters

Two kinds of problems need to be solved within the framework of DS by experimental design. The first is to discover which factors may significantly affect the response of an experiment, and the second is to find factor values that optimize the response [31]. The results of the initial screening and primary risk assessment revealed that the three factors – the initial and final oven temperatures as well as the inlet temperature – greatly affected the retention time and separation of peaks.

The three-level DoE was performed by using JMP software [32] to investigate the optimal levels for the initial oven temperature, the final oven temperature and the inlet temperature. The chromatographic peak areas and the resolution between the peaks were compared under different

Table 3. Target LoQ, reproducibility and measurement uncertainty

Compound	LoQ, mg kg^{-1}	Reproducibility, %	Measurement uncertainty, %
Individual PAHs	< 0.030	< 20	< 30
Aliphatics > C8–C10	< 5	< 20	< 30
Aliphatics > C10–C12	< 5	< 20	< 30
Aliphatics > C12–C16	< 5	< 20	< 30
Aliphatics > C16–C35	< 10	< 20	< 30
Aromatics > C8–C10	< 3	< 20	< 30
Aromatics > C10–C16	< 1	< 20	< 30
Aromatics > C16–C35	< 1	< 20	< 30
Methyl-pyrene/fluoranthene	< 0.5	< 20	< 30
Methyl-chrysene/benz(a)anthracene	< 0.5	< 20	< 30
Individual PCBs	< 0.002	< 20	< 30

instrument parameters. The response of the experiments was the ratio of the peak areas of C35 and C10. The aim was to find whether this ratio was equal to one. When the areas of C10 and C35 are similar, the chromatogram looks symmetrical and the high-mass discrimination effect relatively weak [33]. A total of 21 experiments were conducted using the mixture of aliphatic compounds C10–C35 in the concentration of 25 mg L^{-1} for each compound, and the area ratio of C35/C10 was calculated. Based on these results, it was demonstrated that the required peak area ratio equal to one would be achieved under any conditions within the ranges of 35–40 °C for the initial oven temperature, 335–340 °C for the final oven temperature and 320–325 °C for the inlet temperature. The separation between critical pairs (e.g. benz(a)anthracene and chrysene) and the chromatographic peak areas of PAHs, PCBs and aromatic compounds were evaluated under these conditions, and, furthermore, method validation was conducted.

3.3. Method validation

3.3.1. Extraction and sample preparation

The extraction of PAHs, aliphatics, aromatics and PCBs was based on a modified method of ISO 16703:2011 [34]. Aliquots of 20 g from the soil sample were first extracted in acetone, and then hexane and sodium pyrophosphate (22g L^{-1} $Na_4O_7P_2$) were added. The samples were not dried prior to the analysis. The water content was determined according to ISO 11465:1993 and the analysis results calculated on the dry matter basis [35]. The samples were centrifuged and the upper layer dried with Na_2SO_3 to remove residual water. The dried extract was transferred into the GC vial for the GC-MS analysis.

3.3.2. Instrumental parameters

The GC-MS analysis was carried out under the following conditions: pulsed pressure splitless injection; column flow 1.8 mL min^{-1}; oven temperature programme: initial temperature 40 °C (hold time 3 min); 40 °C min^{-1} to 160 °C; 25 °C min^{-1} to 315 °C (hold time 3.5 min); 40 °C min^{-1} to 340 °C (hold time 9.75 min); interface temperature 320 °C. The analyses were performed in a selected ion monitoring (SIM) mode using two or three ions for each compound for identification [25,29,36].

3.3.3. Results

Table 4 shows that all the results fall within the expected laboratory uncertainty (30%). The reproducibility of the measurements of PCBs, PAHs and aliphatic and aromatic fractions was lower than 20%. The difference between the expected concentrations and those obtained was below 20%. The recovery of PAH compounds ranged from 88 to 112%. For all parent PAHs, a linear calibration curve in the range of 50–5000 ug L^{-1} was obtained; for alkylated PAH compounds it was 0.4–5 mg L^{-1}, for PCBs it was 15–600 ug L^{-1}, and for aliphatic compounds with a coefficient of determination ($R^2 \geq 0.998$) it was 10–50 mg L^{-1}. LoQ, reproducibility, uncertainty and accuracy were assessed using certified reference materials (CRMs): soil CRM BAM-U013b from LGC standards, Teddington, Middlesex, UK; TPH sand 1 (CRM372-100G) from Sigma-Aldrich/Merck, Darmstadt, Germany; SETOC sample from WEPAL, Bennekom, The Netherlands. Two sources of uncertainty were estimated: the uncertainty component from the certified or nominal value and the uncertainty component for bias. Finally, the calculated standard uncertainty was multiplied by a ‘coverage factor’, k = 2, to provide an expanded uncertainty [30].

In the concentration range investigated, the accuracy, precision and uncertainty achieved were satisfactory. All the calculated LoQs were lower than those required by the Swedish legislation. The only limitation of the method was its failure to analyse highly polluted samples. In that case, the samples needed to be diluted and the instrument more frequently maintained.

4. Analytical limits for design space for oil products in the environment

In the literature, one can find studies which deal with the development of the methods of quantification and identification of PAHs [37,38], PCBs [39] and aliphatic and aromatic fractions [40] in soil and also provide the validation of these methods. The methods differ in terms of sample preparation (sample cleanup, filtration, concentration, evaporation), extraction type (Soxhlet extraction, sonication or ultrasonic treatment, mechanical agitation, accelerated solvent extraction (ASE), solid-phase microextraction (SPM)) and analysis/detection (GC-MS, GC-FID, high-performance liquid chromatography (HPLC)). It is outlined that various factors such as sample type, target compound concentration, required sensitivity and available instrumentation influence method choice. Each method has its advantages and disadvantages that the laboratories must consider in setting DS for improving the methods.

In this study, the pros and cons of 10 different GC-MS, GC-FID and HPLC methods, including the one developed in the current study, were compared (Table 5). The cost of extraction techniques, the approximate time spent for the preparation of one sample (including the extraction time) and the greenness of the method were also evaluated. In addition, LoQ, repeatability/reproducibility and recovery rate of the methods were compared.

This comparison of the methods reported in the literature disclosed the absence of a combined method for the simultaneous analysis of PAHs, PCBs and aliphatic and aromatic compounds. The present comparison had to be made between the methods of analysis of PAHs only.

4.1. Analysis techniques used

The most commonly used methods for the PAHs analysis in soils are chromatographic techniques with mass-spectrometry detector, including HPLC and GC-MS. In most cases, GC-MS is the preferred instrument [41]. It is applicable to the majority of parameters and required LoQs. GC-MS was also used in our method due to the availability in the laboratory.

Table 4. The calculated reproducibility, accuracy, uncertainty and LoQ

Compound	Reproducibility, %	Accuracy, %	Uncertainty, %	LoQ, mg kg^{-1}
Naphthalene	2	11	21	0.015
Acenaphthylene	5	10	19	0.014
Acenaphthene	4	12	23	0.016
Fluorene	4	5	12	0.018
Phenanthrene	5	6	10	0.014
Anthracene	7	8	16	0.016
Fluoranthene	3	8	17	0.014
Pyrene	2	10	20	0.015
Benz(a)anthracene	6	7	14	0.019
Chrysene	5	8	16	0.017
Benzo(b)fluoranthene	4	8	15	0.019
Benzo(k)fluoranthene	8	8	17	0.020
Benzo(a)pyrene	2	11	22	0.018
Indeno(1,2,3-cd)pyrene	6	7	14	0.014
Dibenz(a,h)anthracene	4	6	13	0.016
Benzo(g,h,i)perylene	7	9	18	0.016
Aliphatics > C8–C10	10	12	24	5
Aliphatics > C10–C12	9	9	17	5
Aliphatics > C12–C16	8	8	19	5
Aliphatics > C16–C35	10	10	19	10
Aromatics C8–C10	7	9	18	0.9
Aromatics C10–C16	3	3	19	0.9
Methyl-pyrene/fluoranthene	10	13	26	0.2
Methyl-chrysene/benz(a)anthracene	12	14	27	0.1
Aromatics C16–C35	10	12	24	0.9
PCB28	6	9	17	0.001
PCB52	9	10	20	0.001
PCB101	9	12	24	0.002
PCB118	5	5	10	0.002
PCB153	6	13	26	0.002
PCB138	4	4	9	0.002
PCB180	3	11	22	0.002

4.2. Sample size and extraction solvents

Samples of 1 to 60 g were used in different methods. Sample weight is usually selected by the type of solid material and the LoQs to be achieved. The weight of 20 g for a validated method was chosen to get a representative sample with a sufficient LoQ.

The extraction solution volume in the methods under comparison was in the range of 0–150 mL. This is important in determining the greenness of the method. Usually, the extraction solvent used is acetone, hexane or dichloromethane. Dichloromethane can extract many compounds; however, some countries avoid working with dichloromethane as it may cause health issues [42]. The advantage of using acetone and hexane is that it enables more polar compounds to be extracted. The modified method in this study first used acetone alone and then hexane was added. It was confirmed that using solvents in this specific order allows for higher extraction efficiency compared to using solvents individually.

4.3. Calibration standards

Internal standards (ISTDs) or surrogate standards were used in most methods. The number of standards ranged from 3 to 11. External calibration was applied in Methods 2, 4, 7 and 9. The right internal standards can compensate for the low extraction efficiency and reduce the matrix effect. The researchers of the current study used four ISTDs: one for aliphatic compounds, one for high PAHs and PCBs, one for middle PAHs and one for light PAHs.

4.4. Extraction method

Different extraction methods are used. The selection of the extraction method is challenging because the efficiency of the

Table 5. Methods of the PAHs analysis

Method	Reference	Equipment	Extraction type	Number of stages	Time, min	Greenness score	LoQ, mg kg^{-1}	Reproducibility, %	Recovery, %
1 (current study)	N/A	GC-MS	Mechanical	5	60	0.42	0.014–0.020	2–8	88–112
2	42	HPLC-FLD-DAD	Soxhlet	9	1460	0.33	0.001–0.0095	< 5	29.8–96
3	43	GC-MS	ASE	11	40	0.32	0.0001–0.00017	1.3–18.4	80–100
4	44	HPLC-UV-DAD	Sonication	12	190	0.25	0.00005–0.00171	3.34–14.68	82–106
5	45	GC-MS	Soxhlet	9	400	0.22	< 1	1–33	N/A
6	37	GC-MS	Sonication	12	150	0.23	0.00136–0.01186	2–20	68–108
7	46	NTD-GC-FID	SPM	2	40	0.68	0.000001–0.00001	7.3–13.2	92.53–110.75
8	47	GC-MS	Sonication	12	100	0.33	0.0006–0.0054	1.1–13.7	87–128
9	38	HPLC-FLD-DAD	Sonication	7	40	0.34	0.00741–0.45258	< 14	84–87
10	48	GC-MS	Mechanical	5	730	0.45	0.005	< 15	90

extraction of PAHs from soils and sediments is influenced by several factors, such as soil moisture, PAHs content in samples and the texture of soils [43]. The solid-phase microextraction (SPME) with a needle-trap device (NTD) used in Method 7 is a green, solvent-free preparation of the sample; however, packing of sorbents into NTD by this method is a time-consuming process with low repeatability [46]. Soxhlet extraction allows for prolonged and continuous extraction, which can give higher yields compared to other methods [44]. Methods 2 and 5 using Soxhlet extraction require 24 and 6 hours, respectively, to complete a single extraction. Besides being time-consuming, this method uses a large amount of solvent, which is a concern in terms of cost and environmental impact. In Method 5, the extraction solution volume of 120 mL of dichloromethane was used. The accelerated solvent extraction (Method 3) was faster, consumed less solvent and could be easily automated; however, the equipment for accelerated solvent extraction is expensive. In addition, heat-sensitive compounds may still be affected during accelerated solvent extraction [43]. Similarly to Method 10, the present study used mechanical extraction. This extraction is adaptable to different soil types and is easy to implement in a laboratory setting. Mechanical agitation can accelerate the extraction process by facilitating the movement of the solvent through the soil matrix. However, continuous mechanical agitation requires energy, which may increase operational costs.

4.5. Cleanup steps and sample concentration

Different matrices require the application of specific extraction and cleanup steps [45]. As they are mostly related to the maintenance of the instrument, it should be considered whether cleaning is necessary. Some methods include all the steps of preparation, which makes a method complex and time-consuming (Methods 4, 6, 8). In our method, the cleanup stage was found to be unnecessary. Evaporation of extract is usually needed to obtain low LoQ. Almost all methods (2, 3, 4, 5, 6, 8, 9) decreased the amount of extract via rotary evaporation. Method 2 showed the lightest PAHs to be quite sensitive to the evaporation step due to the distinct volatility loss during the evaporation process [42]. The LoQ in our method without evaporation was less than 0.020 mg kg^{-1}. Comparing the threshold values in terms of the content of contaminants in soil established by the Swedish authority, the modified method was sensitive enough. Methods 2, 3, 4, 6, 7, 8 (Table 5) have lower LoQs compared to the current method. However, the cleanup and sample concentration steps make them complicated and time-consuming (with the exception of Method 7 where the number of stages is two). An extra purification step of the samples before the GC analysis could be skipped in the current study, making this part simpler and faster.

5. Assessment of method greenness

The determination of PAH compounds in soil or sediment is a complex analytical procedure that requires significant energy and material inputs. It is also important to assess the environmental friendliness of the methods used. For the latter purpose, the AGREE metric analysis following the 12 principles of green analytical chemistry (GAC) was applied in the present study. The maximum score equal to one represents a methodology that is fully compliant with the 12 principles of GAC [46]. The assessment was performed using the software with an automatically generated graph and an assessment report [47]. Recently, some complementary green assessment tools have become available with the software that can estimate the applicability and functionality of an analytical method [48].

In Table 5, column ‘Greenness score’, the calculated AGREE scores for all the methods under consideration are presented. Using old classical extraction techniques is the reason for the low AGREE analysis scores in all the methods. The Soxhlet extraction and sonication procedures are shown to have explicitly worse scores (less than 0.35), while the mechanical procedures (our method and Method 10) have similar scores (0.42 and 0.45, respectively). One exception is Method 7, where using SPM for the extraction of analytes gives a higher score.

Figure 2 shows the results of the AGREE analysis for our method. The procedure involves an external sample treatment with a reduced number of steps (principle (p) 1) and a soil sample of 20 g (p 2). The measurement is off-line (p 3) and the procedure involves five distinct steps (p 4). The procedure is not automated but involves a miniaturized sample preparation technique (p 5). No derivatization agents are used in the analysis (p 6). Analytical wastes contain 20 g of sample, 20 mL of acetone, 10 mL of hexane and 1 g of Na_2SO_3 (p 7). 43 analytes are determined in a single run, and the sample throughput is two samples per hour (p 8). GC-MS is the most energy-demanding analytical technique (p 9). Some of the reagents (acetone) may originate from bio-based sources (p 10). The procedure requires 10 mL of toxic solvents (hexane) (p 11), while PAHs are persistent and toxic to aquatic life (p 12).

The comparison shows that there is no single ‘best’ method for measuring all types of soil-contaminating PAH compounds. The comparison took into account the cost per sample, energy consumption, time of extraction, the time the analyst spent on sample preparation and the amount of waste, which was crucial in the choice of the appropriate method.

The comparison of the 10 methods revealed the authors’ method to be the most suitable for the intended purpose in every aspect, being also fit for rapid analysis. With all steps of sample preparation included, the time of analysis was approximately one hour. Precision, recovery and peak resolution of the method were highly acceptable and the LoQ complied with the regulatory requirements. The method involves five steps of sample preparation without cleanup and evaporation, making it greener. The greenness score of the present method generally surpasses that of the other methods.

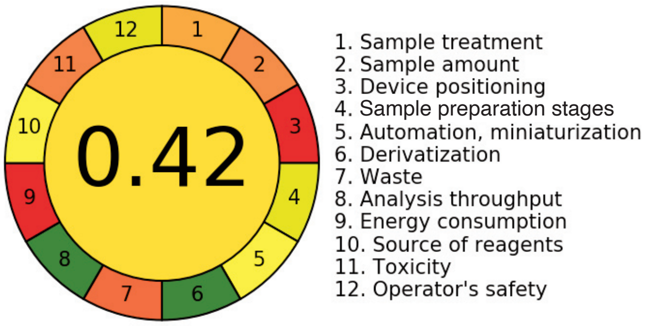


Fig. 2. Results of the AGREE analysis.

6. Conclusions

Design space as an important step in method development for environmental analysis involves a careful study of limits set by authorities as well as the possibilities of existing analytical methods. This provides a strong foundation for the design of experiment, which has helped to identify the optimal instrumental conditions for developing the new method. The simultaneous identification and quantification of 16 polycyclic aromatic hydrocarbons, seven polychlorinated biphenyls, alkylated PAHs and aliphatic compounds in soil involved determining their quality by a design approach in environmental analysis. For sample preparation, the GC-MS analytical technique with the mechanical extraction method was employed. Using a proper GC column with the optimized oven programme enabled achieving sufficient resolution and recovery. Sample preparation was included as part of method development through which an extra purification step of the samples before the GC analysis could be skipped, making this part simpler and faster. By modifying the extraction procedure, it was possible to analyse multiple analytes of PAHs, PCBs and aliphatic and aromatic compounds routinely in a single run with results that comply to Swedish regulations. Decreasing the number of extraction steps in the analysis of a single sample will reduce staff numbers as well as consumable costs. In addition, it reduces the complexity of laboratory organization by diminishing the number of different processes to be followed. Nowadays, it has become almost mandatory to assess the greenness of the developed method and to provide numerical measures for that. Another mandatory part of developing a new method and introducing it to laboratory practice is its validation, which includes an interlaboratory comparison.

The present experiment demonstrated that there is room for method development with improved technical and analytical parameters, which can be used for controlled testing of soil and sediment for PAHs, PCBs and aliphatic and aromatic compounds with reliable accuracy, precision, linearity and recovery rates in the investigated concentration range.

Data availability statement

All data are available in the article.

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References

1. ICH. *ICH Harmonized Tripartite Guideline. Pharmaceutical Development Q8(R2) 2009.* https://database.ich.org/sites/default/files/Q8%28R2%29%20Guideline.pdf (accessed 2024-11-24).
2. ICH. *ICH Harmonized Tripartite Guideline. Pharmaceutical Quality System Q10 2008.* https://database.ich.org/sites/default/files/Q10%20Guideline.pdf (accessed 2024-11-24).
3. ICH. *ICH Harmonized Guideline. Quality Risk Management Q9(R1) 2023.* https://database.ich.org/sites/default/files/ICH_Q9%28R1%29_Guideline_Step4_2023_0126_0.pdf (accessed 2024-11-24).
4. Hakemeyer, C., McKnight, N., St. John, R., Meier, S., Trexler-Schmidt, M., Kelley, B. et al. Process characterization and design space definition. *Biologicals*, 2016, **44**(5), 306–318. https://doi.org/10.1016/j.biologicals.2016.06.004
5. Ng, K. and Rajagopalan, N. Application of quality by design and risk assessment principles for the development of formulation design space. In *Quality by Design for Biopharmaceuticals: Principles and Case Studies* (Rathore, A. S. and Mhatre, R., eds). Wiley, Hoboken, NJ, 2008, 161–174. https://doi.org/10.1002/9780470466315.ch9
6. Manzon, D., Claeys-Bruno, M., Declomesnil, S., Carité, C. and Sergent, M. Quality by design: comparison of design space construction methods in the case of design of experiments. *Chemometr. Intell. Lab. Sys.*, 2020, **200**, 104002. https://doi.org/10.1016/j.chemolab.2020.104002
7. Cocco, P., Ayaz-Shah, A., Messenger, M. P., West, R. M. and Shinkins, B. Target product profiles for medical tests: a systematic review of current methods. *BMC Med.*, 2020, **119**(18). https://doi.org/10.1186/s12916-020-01582-1
8. Jackson, P., Borman, P., Campa, C., Chatfield, M., Godfrey, M., Hamilton, P. et al. Using the analytical target profile to drive the analytical method lifecycle. *Anal. Chem.*, 2019, **91**(4), 2577–2585. https://doi.org/10.1021/acs.analchem.8b04596
9. Kalbe, U., Lehnik-Habrink, P., Bandow, N. and Sauer, A. Validation of European horizontal methods for the analysis of PAH, PCB and dioxins in sludge, treated biowaste and soil. *Environ. Sci. Eur.*, 2019, **31**(29). https://doi.org/10.1186/s12302-019-0211-3
10. Eurachem. *The Fitness for Purpose of Analytical Methods. A Laboratory Guide to Method Validation and Related Topics. 2nd ed., 2014.* https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf (accessed 2024-11-24).
11. ICH. *ICH Harmonized Tripartite Guideline. Validation of Analytical Procedures: Text and Methodology Q2(R1) 1994.* https://database.ich.org/sites/default/files/Q2%28R1%29%20Guideline.pdf (accessed 2024-11-24).
12. Deidda, R., Orlandini, S., Hubert, P. and Hubert, C. Risk-based approach for method development in pharmaceutical quality control context: a critical review. *J. Pharm. Biomed. Anal.*, 2018, **161**, 110–121. https://doi.org/10.1016/j.jpba.2018.07.050
13. Ishikawa, K. *Introduction to Quality Control*. Taylor & Francis, 1990.
14. Fukuda, I. M., Pinto, C. F. F., dos Santos Moreira, C., Saviano, A. M. and Lourenço, F. R. Design of experiments (DoE) applied to pharmaceutical and analytical quality by design (QbD). *Braz. J. Pharm. Sci.*, 2018, **54**(special), e01006. https://doi.org/10.1590/s2175-97902018000001006
15. von Stosch, M., Schenkendorf, R., Geldhof, G., Varsakelis, C., Mariti, M., Dessoy, S. et al. Working within the design space: do our static process characterization methods suffice? *Pharmaceutics*, 2020, **12**(6), 562. https://doi.org/10.3390/pharmaceutics12060562
16. Mas, S., de Juan, A., Tauler, R., Olivieri, A. C. and Escandar, G. M. Application of chemometric methods to environmental analysis of organic pollutants: a review. *Talanta*, 2010, **80**(3), 1052–1067. https://doi.org/10.1016/j.talanta.2009.09.044
17. European Environment Agency. *Down to Earth: Soil Degradation and Sustainable Development in Europe 2000.* https://www.eea.europa.eu/publications/Environmental_issue_series_16/file (accessed 2024-11-24).
18. Lehoux, A. P., Petersen, K., Leppänen, M. T., Snowball, I. and Olsen, M. Status of contaminated marine sediments in four Nordic countries: assessments, regulations, and remediation approaches, *J. Soils Sediments*, 2020, **20**, 2619–2629. https://doi.org/10.1007/s11368-020-02594-3
19. Norwegian Pollution Control Authority. *Guidelines for the Risk Assessment of Contaminated Sites*. https://www.miljodirektoratet.no/globalassets/publikasjoner/klifsft/publikasjoner/andre/1691/ta1691.pdf (accessed 2024-11-24).
20. Ministry of the Environment. *Environmental Damage Fund*. https://ym.fi/en/environmental-damage-fund (accessed 2024-11-24).
21. Danish Environmental Protection Agency. *Environmental Factors and Health. The Danish Experience 2001.* https://www2.mst.dk/udgiv/publications/2001/87-7944-519-5/pdf/87-7944-518-7.pdf (accessed 2024-11-24).
22. Grøn, C., Borling, P., Andersen, L., Cohr, K.-H., Hansen, J. B., Oberender, A. et al. *Olie i jord – forslag til analysemetode og justering af jordkvalitetskriterier, samt grundlag for afskæringskriterier. Miljøprojekt Nr. 1225 2008. Teknologiudviklingsprogrammet for jord- og grundvandsforurening* (*Oil in soil – analysis method proposal and adjustment for soil quality criteria, as well as basis for cutoff criteria. Environmental project No. 1225 2008. Technology development program for soil and groundwater pollution*). https://www2.mst.dk/udgiv/publikationer/2008/978-87-7052-725-5/pdf/978-87-7052-726-2.pdf (accessed 2024-11-24).
23. Nordic Council of Ministers. *Nordic Environmental Permitting Processes.* https://www.researchgate.net/publication/372090099_Title_Nordic_Environmental_Permitting_Processes (accessed
24. Svenska Petroleum Institutet. *Efterbehandling av förorenade bensinstationer och dieselanläggningar 2010* (*Remediation of contaminated gas stations and diesel facilities*). https://drivkraftsverige.se/wp-content/uploads/2023/02/SPBI-rekebh-fororenade-bensinst-dieselanluppdaterad20120129.pdf (accessed 2024-11-24).
25. Naturvårdsverket. *Riktvärden för förorenad mark. Modellbeskrivning och vägledning* (*Guideline values for contaminated soil. Model description and guidance 2009*). https://www.naturvardsverket.se/globalassets/media/publikationer-pdf/5900/978-91-620-5976-7.pdf (accessed 2024-11-24).
26. Eliaeson, K., Kaj, L., Rosenqvist, L., Allard, A-S. and Graae, L. *Påverkan från naturligt organiskt material i GC-MS analyser 2018* (*The influence of natural organic material in GC-MS analyses*). https://www.ivl.se/download/18.694ca0617a1de98f473864/1628417134444/FULLTEXT01.pdf (accessed 2024-11-24).
27. Olivares, L. and Lindén, P. *Instruktion för analys av fraktionen Aromater >C16-C35 2010* (*Instruction for analysis of the aromatic fraction >C16–C35*). https://www.naturvardsverket.se/4a437e/globalassets/vagledning/fororenade-omraden/riktvarden/sis-tk-535-n-012-sis-instruktion-tunga-aromater-c16-c35.pdf (accessed 2024-11-24).
28. Gomes, H. I., Ottosen, L. M., Ribeiro, A. B. and Dias-Ferreira, C. Treatment of a suspension of PCB contaminated soil using iron nanoparticles and electric current. *J. Environ. Manage.*, 2015, **151**, 550–555. https://doi.org/10.1016/j.jenvman.2015.01.015

29. Jurjeva, J. and Koel, M. The chemometric approach to identification of residual oil contamination at former primitive asphalt pavement plants. *Oil Shale*, 2019, **36**(3), 410–430. https://doi.org/10.3176/oil.2019.3.04
30. Magnusson, B., Näykki, T., Hovind, H. and Krysell, M. *Handbook for calculation of measurement uncertainty in environmental laboratories* (*NT TR 537, ed. 3.1) 2012.* https://www.nordtest.info/wp/2012/10/29/handbook-for-calculation-of-measurement-uncertainty-in-environmental-laboratories-nt-tr-537-edition-3-1/ (accessed 2024-11-24).
31. Hibbert, D. B. Experimental design in chromatography: a tutorial review. *J. Chromatogr. B*, 2012, **910**, 2–13. https://doi.org/10.1016/j.jchromb.2012.01.020
32. JMP. https://www.jmp.com/en_us/home.html (accessed 2024-11-24).
33. Wang, X., Cheng, Y., Dong, M., Zhao, L., Chen, L., Feng, T. et al. Research on correcting high-mass discrimination effect of the quadrupole mass spectrometer with dodecane. 2023. *SSRN*, 2023, early access. https://ssrn.com/abstract=4501270 (accessed 2024-11-24).
34. ISO 16703:2011. *Soil quality – Determination of content of hydrocarbon in the range C10 to C40 by gas chromatography.* https://www.evs.ee/en/evs-en-iso-16703-2011 (accessed 2024-11-24).
35. ISO 11465:1993. *Soil quality – Determination of dry matter and water content on a mass basis – Gravimetric method.* https://www.iso.org/standard/20886.html (accessed 2024-11-24).
36. Robbat, A., Jr. and Wilton, N. M. A new spectral deconvolution – selected ion monitoring method for the analysis of alkylated polycyclic aromatic hydrocarbons in complex mixtures. *Talanta*, 2014, **125**, 114–124. https://doi.org/10.1016/j.talanta.2014.02.068
37. Yamada, T. M., Souza, D. A., Morais, C. R. and Mozeto, A. A. Validation of a method for the analysis of PAHs in bulk lake sediments using GC–MS. *J. Chromatogr. Sci.*, 2009, **47**(9), 794–799. https://doi.org/10.1093/chromsci/47.9.794
38. Volk, S. and Gratzfeld-Huesgen, A. *Agilent application solution. Analysis of PAHs in soil according to EPA 8310 method with UV and fluorescence detection 2011.* https://www.agilent.com/cs/library/applications/5990-8414EN.pdf (accessed 2024-11-24).
39. Krzemień-Konieczka, I. and Buszewski, B. Determining polychlorinated biphenyls in soil using accelerated solvent extraction (ASE). *Pol. J. Environ. Stud.*, 2015, **24**(5), 2029–2033. https://doi.org/10.15244/pjoes/41589
40. Sadler, R. and Connell, D. Analytical methods for the determination of total petroleum hydrocarbons in soil. In *Proceedings of the Fifth National Workshop on the Assessment of Site Contamination* (Langley, A., Gilbey, M. and Kennedy, B., eds), 2003, 133–150. https://www.nepc.gov.au/sites/default/files/2022-09/asc-wkshoppaper-09-tphs-sadler-petrol-hydro-soil-200301.pdf (accessed 2024-11-24).
41. Włóka, D. and Placek, A. Study of the effectiveness of the new method for the rapid PAHs analysis in soil. *Acta Innov.*, 2015, **17**, 5–11.
42. Swedish Chemicals Agency. *Swedish rules on chlorinated solvents.* https://www.kemi.se/en/rules-and-regulations/rules-applicable-in-sweden-only/certain-swedish-restrictions-and-bans/chlorinated-solvents (accessed 2024-11-24).
43. Lau, E. V., Gan, S. and Ng, H. K. Extraction techniques for polycyclic aromatic hydrocarbons in soils. *Int. J. Anal. Chem.*, 2010, **2010**, 398381. https://doi.org/10.1155/2010/398381
44. Zhou, W., Zhang, H., Deng, C., Chen, Y., Liao, J., Chen, Z. et al. Solvent-assisted vacuum desorption coupled with gas chromatography-tandem mass spectrometry for rapid determination of polycyclic aromatic hydrocarbons in soil samples. *J. Chromatogr. A*, 2019, **1604**, 460473. https://doi.org/10.1016/j.chroma.2019.460473
45. Song, Y. F., Jing, X., Fleischmann, S. and Wilke, B.-M. Comparative study of extraction methods for the determination of PAHs from contaminated soils and sediments. *Chemosphere*, 2002, **48**(9), 993–1001. https://doi.org/10.1016/S0045-6535(02)00180-7
46. Sajid, M. and Płotka-Wasylka, J. Green analytical chemistry metrics: a review. *Talanta*, 2022, **238**(P2), 123046. https://doi.org/10.1016/j.talanta.2021.123046
47. Pena-Pereira, F., Wojnowski, W. and Tobiszewski, M. AGREE–analytical GREEnness metric approach and software. *Anal. Chem.*, 2020, **92**(14), 10076–10082. https://doi.org/10.1021/acs.analchem.0c01887
48. Manousi, N., Wojnowski, W., Płotka-Wasylka, J. and Samanidou, V. Blue applicability grade index (BAGI) and software: a new tool for the evaluation of method practicality. *Green Chem.*, 2023, **25**, 7598–7604. https://doi.org/10.1039/d3gc02347h

Disainiruumi kontseptsiooni rakendamine saasteainete pinnases määramise meetodi väljatöötamisel

Jelena Jurjeva ja Mihkel Koel

Disainiruum (DS) määrab protsessi sisendite (nt materjalid ja protsessi parameetrid) ja kriitiliste kvaliteediatribuutide vahelise seose. Kvaliteedijuhtimise seisukohast on see kontseptsioon väga oluline, sest DSis töötamist ei peeta muudatuseks. Disainiruumi rakendamine on vajalik samm keskkonnaanalüüsi meetodite väljatöötamisel, et arvestada saasteainete piirnormidega ja toetada olemasolevate analüüsimeetodite arendamist. Selles töös rakendati disainiruumi kontseptsiooni naftareostusest pärinevate ühendite analüüsimeetodi väljatöötamiseks, keskendudes polütsüklilistele aromaatsetele süsivesinikele, polüklooritud bifenüülidele ning erinevatele alifaatsetele ja aromaatsetele fraktsioonidele. Sihtühenditele rakendatud disainiruumi kontseptsioon koosneb järgmistest etappidest: meetodi kriitiliste parameetrite tuvastamine, tulemuslikkuse hindamine, vastuvõetavate tööpiiride määramine, tingimuste optimeerimine, meetodi valideerimine, seire ja kontroll. Meetodi arendus hõlmas ka muudatusi proovide ettevalmistuses, mis lihtsustas ja kiirendas vastavat osa mõõtmisprotsessis. Lisaks võimaldas disainiruum tuvastada olulisi tegureid ning optimeerida neid statistilise analüüsi ja katsete planeerimise meetodite abil.