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### Quantification of naphthalene in soil using solid-phase microextraction, gas-chromatography with mass-spectrometric detection and standard addition method

**Abstract:** Development of simple, fast and accurate methods for quantification of volatile organic compounds (VOCs) in soil samples is important for providing greater efficiency of analytical laboratories in Kazakhstan and other developing countries. Naphthalene is a model polycyclic aromatic hydrocarbon (PAH), belonging to a group of compounds of significant concern due to environmental impact. Solid-phase microextraction (SPME) is an optimal method for solvent-free automated sample preparation for determination of VOCs in environmental samples. In this work, the method for quantification of naphthalene in soil based on headspace SPME, gas chromatography with mass spectrometric detection, and standard addition calibration was developed. The parameters of SPME and sample equilibration after spiking with standards were optimized for better control of the soil matrix effect. The SPME temperature 80 °C provided the greatest accuracy of naphthalene responses for soils with different matrix and humidity. Equilibration of soil samples after spiking with standards for 6 h at 80 °C provided stabilization of responses in soils with different matrix and water content. The greatest accuracy and precision were achieved after equilibration of the samples for 8 h. The method provides recoveries of 105-119% in the concentration range 0.01-0.1 ng g<sup>-1</sup> with detection limit 0.001 ng g<sup>-1</sup>. The developed method was applied for quantification of naphthalene in real soil samples collected in Almaty, Kazakhstan. The measured concentration of naphthalene in real soil samples varied in the range of 1.4 to 47 ng g<sup>-1</sup>. In five out of ten collected soil samples concentration exceeded a maximum permissible concentration of 15 ng g<sup>-1</sup>.

**Key words:** naphthalene, soil analysis, solid-phase microextraction, standard addition, gas chromatography

#### Introduction

Naphthalene is a ubiquitous pollutant found in many environmental samples of air, water, soil. It is a confirmed carcinogen and mutagen linked to hemolytic anemia and cataracts in humans [1,2]. Along with other polycyclic aromatic hydrocarbons (PAHs), naphthalene can be released into the environment during transportation, storage, and use of crude oil and oil products [2]. The soil is often the main sink of PAHs spills.

Standard methods for quantification of naphthalene in soil require waste-generating, costly and time-consuming sample preparation using toxic organic solvents (e.g., acetonitrile, acetone, chloroform, diethyl ether, methanol). The extraction of an analyte from soil is followed by purification of the extracts by passing through adsorbent (e.g., aluminum oxide, silica gel) and an evaporative concentration [3–5],

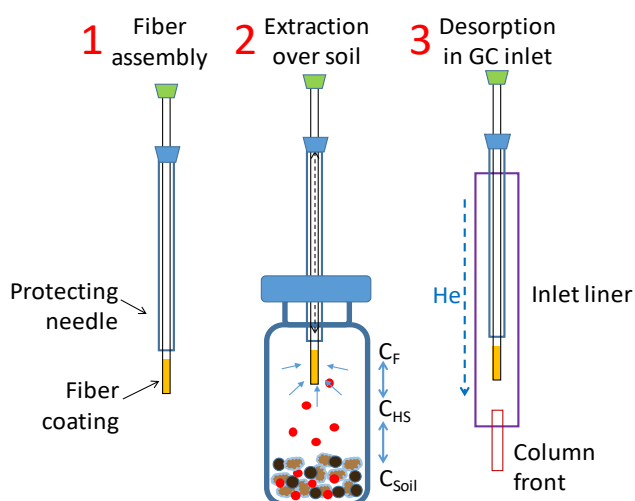
which can result in the emission of toxic compounds to an environment.

The most promising “green” method of sample preparation for the determination of volatile and semi-volatile organic compounds (VOCs and SVOCs) in soils is solid-phase microextraction (SPME) [6]. SPME combines sampling, extraction, concentration, and purification steps into one easily automated operation. During SPME, VOCs are transferred onto the polymer coating from a headspace (HS) above a soil sample. Extracted analytes are then desorbed in an inlet of a gas chromatograph for analysis (Figure 1). This method does not require organic solvents and provides an ideal combination of simplicity and sensitivity.

Available SPME-based methods for quantification of naphthalene and other PAHs in soil include preliminary extraction with a surfactant solution [7] or an organic solvent [8], while SPME is conducted in direct-immersion (DI) mode. For headspace SPME,

extraction is conducted with simultaneous heating of the sample and cooling of a fiber coating (cold-fiber SPME) [9–11], or at low-pressure conditions (vacuum-assisted HS-SPME) [12]. The main limitations of CF-SPME and vacuum-assisted HS-SPME are the complexity of method instrumentation and its automation, along with no commercial availability.

The accuracy of SPME-based quantification of VOCs and SVOCs in soil is affected by a matrix effect, which results in variable extraction efficiencies of analytes from samples having different physico-chemical properties. To overcome the matrix effect, two main approaches are used: control of the matrix effect by obtaining a calibration plot for each type of soil; and a decrease of the matrix effect by increasing the extraction temperature, addition of water, or using cold-fiber SPME [13]. The main difficulty in using the first approach is a long and often inefficient equilibration of samples after their spiking with standards [14,15].



**Figure 1** – Solid-phase microextraction of organic compounds from soil

Note: Stages of SPME: 1 – Fiber holder (before extraction); 2 – extraction; 3 – desorption;  $C_F$  – analyte concentration in fiber;  $C_{HS}$  – analyte concentration in headspace;  $C_{Soil}$  – analyte concentration in soil

In this work, a fast, green, and accurate method for quantification of naphthalene in soil samples using headspace (HS) SPME, GC with MS detection, and the standard addition calibration was developed. Parameters of HS-SPME were optimized to provide the best accuracy of naphthalene responses for soils with different matrix and humidity. Soil equilibration

temperature and time after spiking with naphthalene standard were optimized. The developed method was successfully applied for the analysis of real soil samples.

## Experimental

### Reagents, materials, and samples

Naphthalene (99%) was purchased from Meryer (China). Methanol (HPLC grade) purchased from AppliChem (Germany) was used for the preparation of standard solutions. SPME fiber – 100  $\mu\text{m}$  polydimethylsiloxane (PDMS) was purchased from Supelco (USA). Soil sampling and calibration were performed in 20-mL crimp-top headspace vials (HTA, Italy) with PTFE/silicone septa (Zhejiang Aijiren Technology Co., China). All vials and septa were washed with distilled water and pre-conditioned at 140  $^{\circ}\text{C}$  for 2 h before analysis.

Two different soil types were used: clay and chernozem having humus content 0.90 and 45%, respectively. These soils will be referred hereinafter as soils with ‘low’ and ‘high’ humus content (LHC and HHC), respectively. Both soils were collected near Almaty, Kazakhstan. Soils were cleaned from possible naphthalene residues and water by heating in a drying furnace at 150  $^{\circ}\text{C}$  for 6 h.

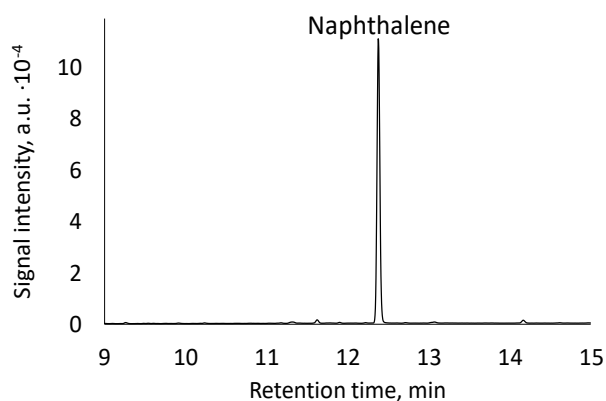
### Parameters of gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analyses were performed on the 6890N/5973N system (Agilent, USA) equipped with a Combi-PAL autosampler (CTC Analytics, Switzerland). Separation was conducted using a polar 60 m x 0.25 mm DB-WAXetr (Agilent, USA) column with a 0.50  $\mu\text{m}$  film thickness at constant helium (>99.995%, Orenburg-Tehgas, Russia) flow 1.0 mL  $\text{min}^{-1}$ . The oven temperature was programmed from 80  $^{\circ}\text{C}$  to 160  $^{\circ}\text{C}$  with the heating rate 20  $^{\circ}\text{C} \text{ min}^{-1}$ , and further heating to 240  $^{\circ}\text{C}$  (held for 3 min) with the heating rate 10  $^{\circ}\text{C} \text{ min}^{-1}$ . GC run time was 15 min (sample chromatogram is shown in Figure 2). Temperatures of the ion source, quadrupole and MS interface were 230, 150 and 240  $^{\circ}\text{C}$ , respectively. Detection was conducted using electron impact ionization at 70 eV in selected ion monitoring (SIM) mode at  $m/z$  of 128.

### Headspace solid-phase microextraction (HS-SPME) procedure

Soil samples weighing 1.00 g were placed into 20-mL crimp-top headspace vials and spiked with 10

$\mu\text{L}$  of standard solution of naphthalene ( $C = 10 \text{ ng } \mu\text{L}^{-1}$ ). Vials were placed into the agitator of the autosampler and incubated at a preset temperature for 10 min. The headspace extraction was conducted for 5 min at a preset temperature of the agitator of the autosampler using  $100 \text{ }\mu\text{m}$  PDMS fiber. After extraction, the analyte was completely desorbed from the SPME fiber in the GC inlet at  $240 \text{ }^\circ\text{C}$  for 60 s.



**Figure 2** – Selected ion monitoring ( $m/z$  128) chromatogram obtained after SPME-GC-MS analysis of soil spiked with naphthalene standard solution

#### *Study of the effect of soil water content and extraction temperature on intensity and precision of naphthalene responses*

Soils with water content of 0%, 25%, and 50% were prepared by addition of 0, 250 or 500  $\mu\text{L}$  of water to 1.00 g soil samples pre-spiked with 10  $\mu\text{L}$  of a standard solution of naphthalene ( $C = 10 \text{ ng } \mu\text{L}^{-1}$ ). Studied extraction temperatures were 60, 70 and  $80 \text{ }^\circ\text{C}$ . Responses were measured in three replicates.

#### *Study of the effect of temperature and time on equilibration of naphthalene in soils with different humus and water content*

Vials with HHC and LHC soils of the different water content (0, 25, and 50%) were placed into the agitator of the autosampler and equilibrated at 60, 70, and  $80 \text{ }^\circ\text{C}$ . The HS-SPME was conducted immediately after spiking and every 2 h until stabilization of the naphthalene responses. All experiments were conducted in three replicates.

#### *Method validation*

Model samples of HHC and LHC soils with concentrations of naphthalene 10 and  $100 \text{ ng g}^{-1}$  were prepared by spiking 1.00 g of soil with 10  $\mu\text{L}$  of standard solutions with concentrations of naphthalene 1

and  $10 \text{ ng } \mu\text{L}^{-1}$ , respectively. After spiking, the model samples were held for 48 h at  $60 \text{ }^\circ\text{C}$ . Calibration standards for standard addition calibration were obtained by spiking 1.00 g of model samples with 10  $\mu\text{L}$  of standard solutions with concentrations of naphthalene 0, 0.5, 2.0, 5.0, and  $10 \text{ ng } \mu\text{L}^{-1}$ . After spiking with standards, the soils samples were equilibrated for 8 h at  $60 \text{ }^\circ\text{C}$ . Slope factors of calibration plots, measured concentrations, and their standard deviations were calculated using least squares method and standard addition approach.

## Results and discussion

### *Optimization of HS-SPME of naphthalene from soil samples*

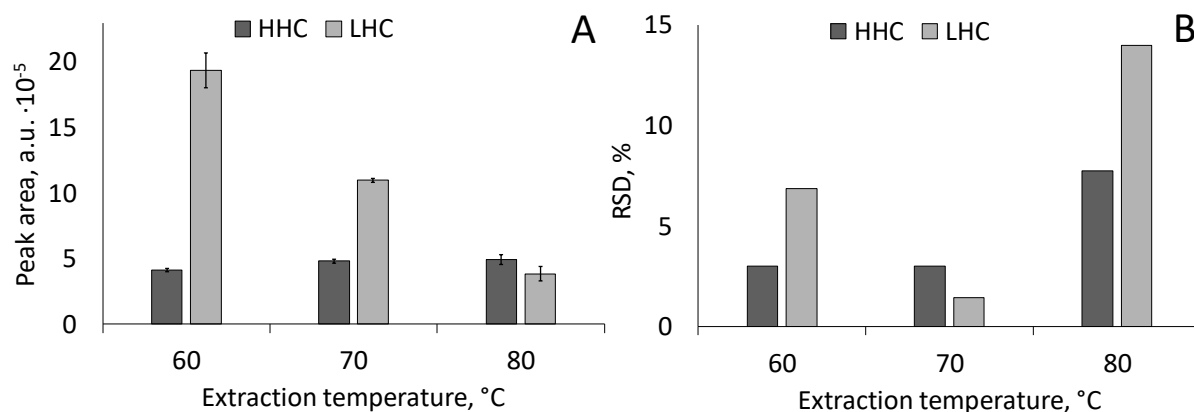
The precision of the analytical responses has a significant effect on the accuracy of quantification of analytes using the standard addition method. To achieve the acceptable accuracy at high standard deviation, an increase in the number of calibration standards is required, which results in the increase of the cost of the analysis. The aim of this experiment was to study the effect of extraction temperature from soil samples with different matrices on the intensity and precision of naphthalene responses.

Naphthalene extraction effectiveness from soils with low humus content (LHC) decreased with increasing extraction temperature from 60 to  $80 \text{ }^\circ\text{C}$  (Figure 3A). The increase of the extraction temperature from 60 to  $70 \text{ }^\circ\text{C}$  resulted in the decrease of the response by 50%. In soils with high humus content (HHC), such increase in the extraction temperature resulted in the increase of the response by 10 to 14%. At  $80 \text{ }^\circ\text{C}$ , responses of naphthalene for soils with low and high humus content became practically similar meaning that this temperature allows such analysis at minimum matrix effects. However, the increase of the extraction temperature to  $80 \text{ }^\circ\text{C}$  resulted in the increase of the relative standard deviations (RSDs) of naphthalene responses in soils with different matrix (Figure 2B). The optimal temperature of naphthalene extraction from soil samples for minimization of the matrix effect is  $80 \text{ }^\circ\text{C}$ . However, optimization of sample equilibration after spiking with standards is required in order to provide proper precision and accuracy of responses.

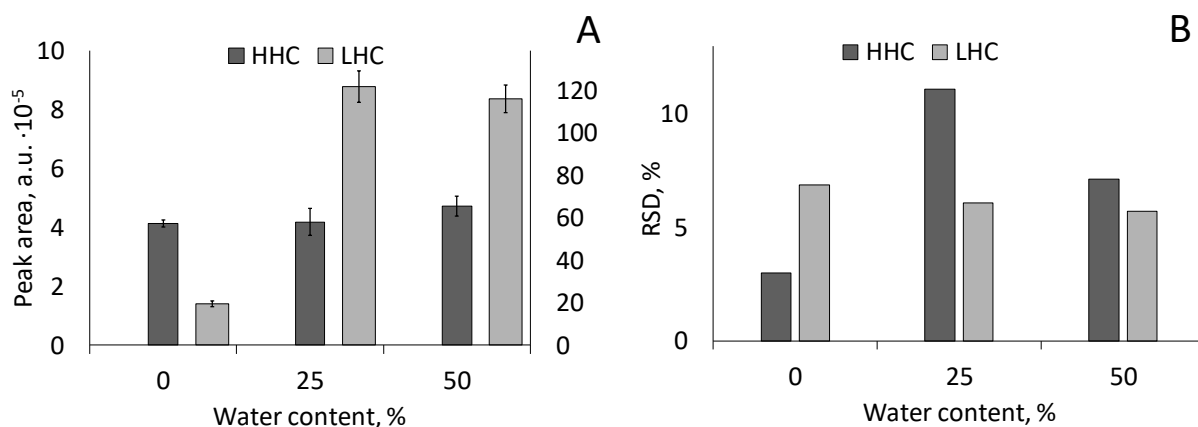
The increase of water content of soil did not affect naphthalene extraction effectiveness from soils with high humus content (Figure 4). RSDs of responses

increased with the increase of soil humidity. At 0% humidity, the RSDs of naphthalene in high humus

content soil (HHC) was 2.9%, at water content 25% and 50%, RSDs were 3.2 and 7.1%, respectively.



**Figure 3** – Effect of SPME temperature on intensity (A) and precision (B) of the naphthalene responses from soils with high and low humus content. *Note:* water content: 0%, extraction time 5 min, C = 100 ng g<sup>-1</sup>



**Figure 4** – Effect of water content on intensity (A) and precision (B) of the naphthalene responses for soils with HHC (on the primary axis) and LHC (on the secondary axis). *Note:* extraction temperature 60 °C, extraction time 5 min, C = 100 ng g<sup>-1</sup>

In soils with low humus content, the increase in soil humidity up to 25 and 50% resulted in the increase of responses by the factor of ~7. The addition of water to the soil, therefore, increases the difference in the effectiveness of naphthalene extraction from soils with different matrix. The increase of soil water content leads to a more efficient desorption of naphthalene from soils with low humus content and can lead to analyte losses during the sample preparation for calibration using the standard addition method. Thus, for SPME-GC-MS determination of naphthalene by the standard addition method, it is undesir-

able to introduce water additives into the samples before extraction.

#### *Equilibration of naphthalene in soils of different type and water content*

Equilibration of soils after spiking by standards is required in order to achieve high accuracy using the standard addition method [14,15]. Equilibration of spiked soil samples ensures the same extraction efficiency of analytes already present in the sample and spiked standards.

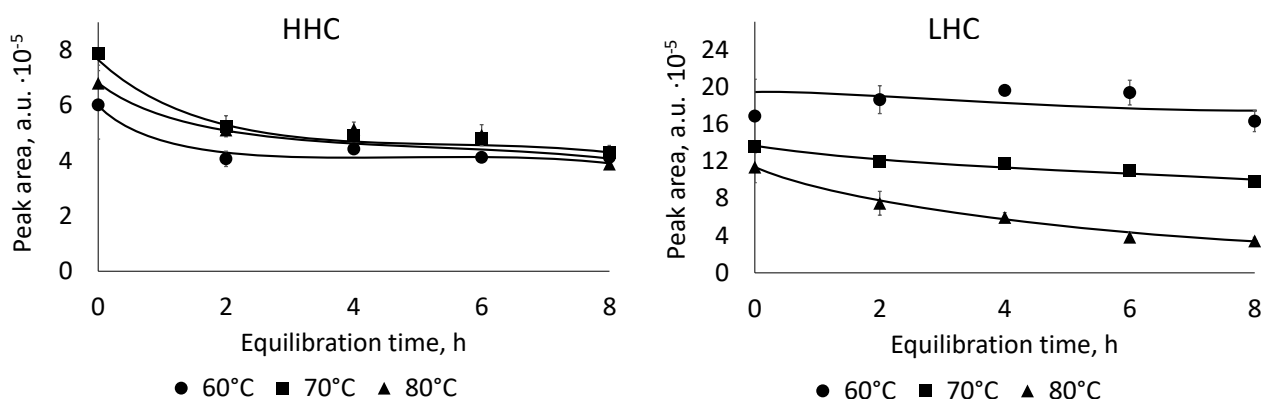
Stabilization of naphthalene responses for soils with high humus content proceeded with the decrease

of their intensity (Figure 5). At all studied equilibration temperatures, the stabilization of the naphthalene responses for the HHC soil was achieved within 2 h. When equilibrated for 8 h, the naphthalene responses in all parallel experiments and at different equilibration and extraction temperatures were equalized.

For LHC soil equilibrated at 60 °C, the signal was fluctuating in the range of standard deviation during 6 h without a decrease of the responses, decrease of the responses was observed only after 6 h of equilibration. When equilibrating LHC soil at

70 and 80 °C, the responses after 8 h of equilibration decreased by 20 and 70%, respectively, showing that increase of temperature enhances equilibration of LHC soils. The rate of equilibration of LHC soils after spiking with naphthalene is lower than for HHC soils, and equilibration was observed only after 6 to 8 h at 80 °C.

RSDs of responses of naphthalene decreased as the equilibration time increased at all studied temperatures (Figure 6). The lowest RSDs were obtained at equilibration temperature 70 °C.

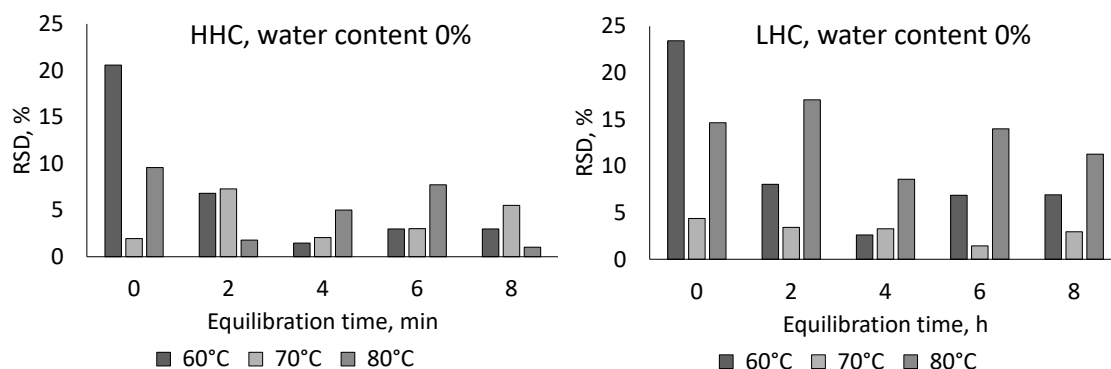


**Figure 5** – Effect of temperature and time of equilibration on responses of naphthalene from soils with HHC and LHC.

Note: water content 0%, extraction time 5 min,  $C = 100 \text{ ng g}^{-1}$

Soil humidity affects equilibration of soil samples after spiking with naphthalene (Figure 7). In HHC

soils the stabilization of the naphthalene responses was observed in 6 h for all studied water contents.



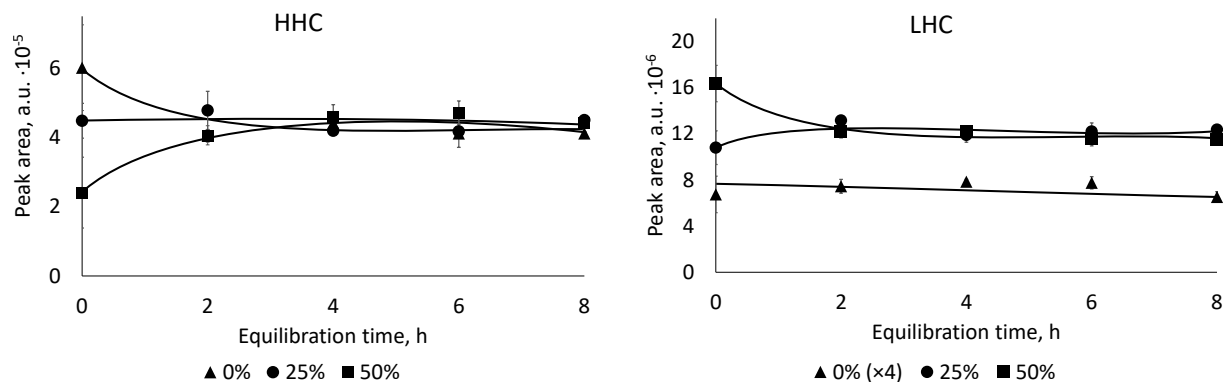
**Figure 6** – Effect of equilibration time on precision of naphthalene responses from soil samples

With the increase of water content in HHC soil, the increase of naphthalene responses during equilibration was observed. When the HHC soil was equilibrated in a wide range of water contents (0 to 50%) for 6 h or more, the responses of naphthalene were

equalized, and the precision of the results increased. In LHC soils with 0% water content, the equilibration was not observed at all studied equilibration times. For LHC soils with water contents 25 and 50%, equilibration was achieved in 4 h (Figure 7). Thus, the

optimum temperature of soil equilibration after spiking with naphthalene standards is 80 °C. Stabilization of responses in all types of soil and water contents

was achieved after 6 h of equilibration. The optimal equilibration time providing best combination of accuracy and precision of the results was 8 h.



**Figure 7** – Effect of soil water content on the equilibration of soils with different matrix after spiking with naphthalene at 60 °C

#### *Validation of the method*

The developed method was applied for quantification of naphthalene in model soil samples with concentrations 10 and 100 ng g<sup>-1</sup>. The method provided good linearity of the calibration plots obtained using standard addition approach and least squares method with coefficients of determination ranging from 0.968 to 0.998 for concentrations 1 to 250 ng g<sup>-1</sup> (Table 1). Higher coefficients of determination of the calibration plots were obtained for LHC soils. Method detection limit is 1 ng g<sup>-1</sup>.

The accuracy of quantification of naphthalene in soils with a high concentration (100 ng g<sup>-1</sup>) is higher than in soils with a low concentration (Table 1). Deviations from the spiked concentrations were 10 to 18%. In soils with a low concentration of naphthalene, deviations from the spiked concentrations were 5 to 29%. The greatest deviations between measured and spiked concentrations were obtained in LHC soils with low analyte concentration. This can be explained by the low affinity of naphthalene to the soil, which leads to its less homogeneous distribution in the soil.

**Table 1** – Results of quantification of naphthalene in model samples using the developed method

Soil type	Spiked concentration (ng g <sup>-1</sup> )	Measured concentration (ng g <sup>-1</sup> )	Recovery (%)	R <sup>2</sup>
HHC	10	10.5±0.9	105±9	0.968
LHC	10	12.9±1.7	129±13	0.993-0.998
HHC	100	110±11	110±10	0.969-0.982
LHC	100	118±11	118±9	0.989-0.993

#### *Application of the developed method on real soil samples*

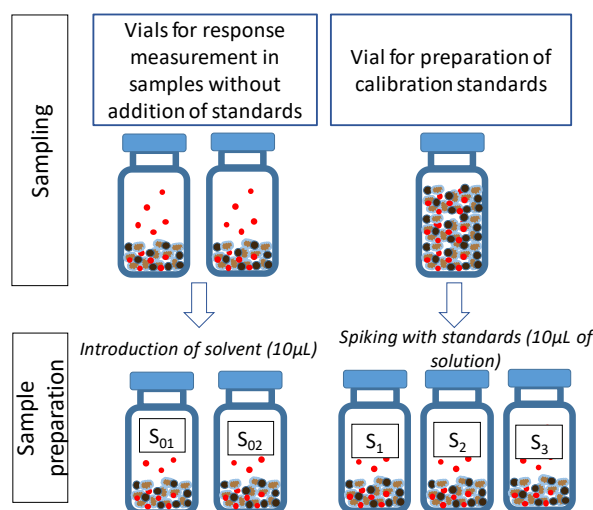
The developed method was applied for the analysis of ten soil samples collected in different locations in Almaty, Kazakhstan (map of sampling locations is shown in [16]) in September 2017. Sampling and

sample preparation were optimized to minimize the loss of analytes (Figure 8). Samples for response measurement were collected separately into pre-weighed vials and analyzed from sealed vials without any sample preparation. Samples for preparation of calibration samples were collected at the same

location and prepared as described in the optimized method.

Naphthalene was detected in all collected samples (Table 2). In five samples, concentrations of naphthalene were close to or higher than the maximum permissible concentration (MPC, 15 ng g<sup>-1</sup>). Concentrations of naphthalene in samples 4 and 5 collected in park area and gas station territory, respectively, were three and two times higher than MPC.

The slope factors of obtained calibration plots varied in the range 1991-12842. This significant difference in slope factors could be caused by different humidity of the samples, as it was shown previously that increase of water content results in significant response differences of naphthalene from soils. These results imply the importance of matrix control by standard addition calibration, since external standard calibration will not provide acceptable accuracy of quantification.



**Figure 8** – Scheme of soil sampling and analysis using the developed method

**Table 2** – Naphthalene concentrations determined in real soil samples using of the developed method

#	Measured concentration (ng g <sup>-1</sup> )	RSD (%)	Slope factors of calibration plots	R <sup>2</sup>	Location description
1	18 ± 3	17	2785	0.972	Private residential sector
2	13 ± 2	17	1991	0.977	Near the roadway
3	47 ± 5	11	3823	0.991	Park area
4	31 ± 3	9	6674	0.998	Gas station area (Gas Energy)
5	15 ± 2	13	8615	0.984	Residential area
6	10.2 ± 1.2	12	12842	0.986	Student residential area (KazNU)
7	1.4 ± 0.2	15	11745	0.995	Near the major roadway (Timiryazev st.)
8	16 ± 3	22	9785	0.963	Park area next to roadway
9	2.1 ± 0.4	17	11916	0.982	Gas station area (KazMunaiGaz)
10	8.9 ± 1.4	16	9552	0.993	Near the major roadway (Gagarin st.)

## Conclusion

Fast, green, and automated method for quantification of naphthalene in soil samples based on solid-phase microextraction and gas-chromatography with mass-spectrometric detection was developed and tested. Optimal temperature of SPME providing best matrix effect minimization is 80 °C. The optimal temperature for soil equilibration after spiking with naphthalene standards is 70 °C. Stabilization of responses in all types of soil and water contents at 80 °C is achieved in 6 h. The greatest accuracy

and precision are achieved after equilibration of the samples for 8 h. The uncertainty in the determination of naphthalene in soils with concentration higher than 100 ng g<sup>-1</sup> does not exceed 18%; for soils with a concentration higher than 0.01 ng g<sup>-1</sup>, uncertainty is less than 29%.

The developed method provides simple and fast quantification of naphthalene with sufficient accuracy without using toxic organic solvents. The method can be applied in environmental analytical laboratories for quantification of naphthalene in different soils.

## Acknowledgments

This work was conducted under the project 3661/GF4 “Development and implementation of “green” methods for determination of organic pollutants in soils” and Ph.D. project of Dina Orazbayeva both funded by the Ministry of Education and Science of Kazakhstan.

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