Determination of nitrogen-containing pesticides in soil using vacuum-assisted headspace solid-phase microextraction

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Pesticides are one of the largest groups of environmental pollutants used to protect agricultural plants from different pests and weeds. Soil is the initial area of accumulation of pesticides after their release into the environment. Determination of pesticides in soil is complicated by matrix effects and laborious sample preparation which generally involves the use of large amounts of organic solvents. Development of accurate green analytical methods for determination of pesticides in soil is an urgent task in environmental and analytical chemistry.

In this study a method based on vacuum-assisted headspace solid-phase microextraction (Vac-HSSPME) coupled with gas chromatography-mass-spectrometry (GC-MS) was developed for the quantification of nitrogen-containing pesticides in soil samples. The pesticides atraton, simazine, atrazine, propazine, diazinon, metribuzin, prometryn, and oxyfluorfen were target analytes. The effects of water addition, reduced pressure, salting-out and pH adjustment on the extraction efficiency of target pesticides from soil were studied.

Using Vac-HSSPME, the increase in the responses for all target pesticides by 3-7 times compared to ambient-pressure HSSPME was observed. Addition of water resulted in 2 to 380 times increase of the peak areas of analytes obtained using Vac-HSSPME. Optimum Vac-HSSPME performance was achieved using 60 min extraction at 60 °C. The proposed method can be recommended for quantification of atraton, atrazine, propazine, diazinon, prometryn, and oxyfluorfen in soil. Under optimum conditions the weighted linear regressions with $R^2 > 0.949$ were obtained for most analytes in the concentration range 25-200 ng/g. The limits of detection and quantification ranged from 0.1 to 4 ng/g, and from 0.4 to 12 ng/g, respectively.

Keywords: headspace solid-phase microextraction; vacuum-assisted headspace solid-phase microextraction; pesticides; soil analysis; gas chromatography; mass spectrometry.

Бу фазалы қатты фазалы микроэкстракцияның қолдануымен топырақтағы азотты пестицидтерді анықтау

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Пестицидтер – ауылшаруашылық өсімдіктерін әртүрлі зиянкестер мен арамшөптерден қорғау үшін қолданылатын қоршаған ортаны ластаушы заттардың ең үлкен топтарының бірі. Пестицидтердің қоршаған ортаға енгеннен кейін жиналуының бастапқы аймағы – топырақ болып табылады. Топырақтағы пестицидтердің құрамын анықтау матрицаның әсерімен, және әдетте органикалық еріткіштердің көп мөлшерін қолдануды талап ететін ұзақ үлгілерді дайындаумен қиындайды. Топырақтағы пестицидтерді анықтаудың дәл әрі жасыл аналитикалық әдістерін әзірлеу – экологиялық және аналитикалық химия саласындағы өзекті мәселе.

Бұл зерттеуде топырақ үлгілеріндегі азотты пестицидтерді сандық анықтау үшін вакуумды қатты фазалы микроэкстракция (Вак-ҚФМЭ) және газ хроматографиясымен масс-спектрометриямен (ГХ-МС) негізіндегі әдістеме әзірленді. Талданатын мақсатты заттар: атратон, симазин, атразин, пропазин, диазинон, метрибузин, прометрин, оксифлуорфен. Суды қосу, қысымды төмендету, тұздаттыру және pH өзгертудің топырақтан мақсатты пестицидтерді экстракциялау тиімділігіне әсері зерттелді.

Вак-ҚФМЭ қолданған кезде атмосфералық қысымдағы ҚФМЭ-мен салыстырғанда барлық мақсатты пестицидтер көрсеткіштерінің 3-7 есе артуы байқалды. Судың қосылуы Вак-БФҚФМЭ көмегімен алынған талданатын заттардың шың аудандары 2-тен 380 есе өсуіне әкелді. Вак-БФҚФМЭ 60 °C температурада 60 мин экстракцияны қолдану арқылы оңтайлы өнімділігіне қол жеткізілді. Оңтайлы көрсеткіштермен талданатын заттардың көпшілігі үшін 25-200 нг/г концентрация диапазонында R^2>0,949-мен өлшенген сызықтық регрессиялар алынды. Анықтау және сандық шектеулер сәйкесінше 0,1-дан 4 нг/г-ға дейін және 0,4-ден 12 нг/г-ға дейін өзгерді. Ұсынылған әдістеме топырақтағы атратон, атразин, пропазин, диазинон, прометрин және оксифторфенді сандық анықтау үшін ұсынылады.

Түйін сөздер: қатты фазалы микроэкстракция; вакуумды қатты фазалы микроэкстракция; пестицидтер; топырақты талдау; газды хроматография; масс-спектрометрия.

Определение азотсодержащих пестицидов в почве с использованием вакуумной парофазной твердофазной микроэкстракции

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Пестициды являются одной из крупнейших групп загрязнителей окружающей среды, используемых для защиты сельскохозяйственных растений от различных вредителей и сорняков. Почва является первоначальным ареалом накопления пестицидов после их попадания в окружающую среду. Определение пестицидов в почве осложнено матричными эффектами и трудоемкой пробоподготовкой, которая, как правило, включает в себя использование больших объемов органических растворителей. Разработка точных «зеленых» аналитических методик определения пестицидов в почве является актуальной задачей в области экологической и аналитической химии.

В данном исследовании был разработан метод на основе вакуумной парофазной твердофазной микроэкстракции (Вак-ПТФМЭ) в сочетании с газовой хроматографией с масс-спектрометрическим детектированием (ГХ-МС) для количественного определения азотсодержащих пестицидов в образцах почвы. Атратон, симазин, атразин, пропазин, диазинон, метрибузин, прометрин и оксифлуорфен были целевыми аналитами. Было изучено влияние добавления воды, снижения давления, добавления соли и изменения pH на эффективность экстракции целевых пестицидов из почвы.

При использовании Вак-ПТФМЭ наблюдалось увеличение откликов всех целевых пестицидов в 3-7 раз по сравнению с ПТФМЭ при атмосферном давлении. Добавление воды привело к увеличению площадей пиков аналитов, полученных с использованием Вак-ПТФМЭ, от 2 до 380 раз. Оптимальные условия Вак-ПТФМЭ были достигнуты при экстракции в течение 60 мин при 60 °C. При оптимальных условиях были получены взвешенные линейные регрессии с R^2>0,949 для большинства аналитов в диапазоне концентраций 25-200 нг/г. Пределы обнаружения и количественного определения варьировались от 0,1 до 4 нг/г и от 0,4 до 12 нг/г, соответственно. Предлагаемый метод может быть рекомендован для количественного определения атратона, атразина, пропазина, диазинона, прометрина и оксифлуорфена в почве.

Ключевые слова: твердофазная микроэкстракция; вакуумная твердофазная микроэкстракция; пестициды; анализ почвы; газовая хроматография; масс-спектрометрия.

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1. Introduction

Pesticides are substances designed to destroy, control, kill, repel any undesirable living organisms hindering agricultural activities. Agricultural workers and households are mostly vulnerable to pesticides causing diseases due to frequent and high-dose exposures [1]. Workers at pesticide manufacturing companies are at the highest risk of serious illnesses since they deal with chemicals, raw materials and toxic solvents [2]. Often people are exposed to not a single pesticide, but a complex mixture which makes the effect on health even more harmful [3]. Pesticides not only accumulate in the crop's parts, but also migrate through the air, soil and water, which causes the pollution of ecosystems [4]. Pesticides evaporate mainly from soil and cause contamination of every part of the biosphere [5]. These days pollution of soil by pesticides is one the biggest concerns, because rapid rise of the agricultural activities leads to tremendous soil contamination with these compounds [6]. Moreover, pesticides accumulate in soil for many years and subsequently migrate downward causing a pollution of groundwater [7]. They also reduce the activity of soil enzymes, thereby decreasing its fertility and productivity [8]. Certain pesticides transformation products are far more toxic and mobile in the soil than the parent compounds and therefore tend to be even more dangerous to the environment [9].

Shifting toward greener sample preparation approaches is the main trend in the field of pesticides analysis in soil [9]. Solid-phase microextraction (SPME) is one of the most effective green methods for extraction of pesticides in soil that does not require the use of organic solvents, eliminating potential human health and environmental hazards. The principle of SPME is based on extraction of analytes from the headspace of the sample onto sorbent fiber coating followed by desorption in the inlet of gas chromatograph (GC). Compared to standard methods of sample preparation used in pesticide analysis, headspace solid-phase microextraction (HS-SPME) offers solvent-free, fast and simple extraction and purification unified into the single process. The combination of HS-SPME with gas chromatography-mass spectrometry (GC-MS) has been successfully applied for quantification of pesticides in different environmental and biological samples [10–12].

SPME-based quantification of pesticides in soil is complicated by matrix effects and low volatility of analytes, which leads to longer extraction times. In this study, we propose to overcome these limitations of HS-SPME by using a so-called vacuum-assisted solid-phase microextraction (Vac-HSSPME) approach. In 2012, Psillakis et al. developed a new method based on Vac-HSSPME, in which air-evacuation prior to extraction improved the extraction rates of chlorophenols from water samples [13]. Reduced system pressure facilitates the extraction due to higher diffusion rate of molecules, resulting in increased extraction efficiency of compounds with low Henry's law constants within short extraction times [14]. Hence, the Vac-HSSPME is a promising approach for the analysis of semi-volatile organic compounds such as pesticides in complex matrices.

Organonitrogen pesticides are one the most frequently used and detected in soil in the last decades [9], creating an incentive for introducing new efficient and green analytical methods for their quantification. Key properties of target pesticide and their maximum permissible levels in soil are listed in Table 1.

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Table 1 – Key properties of target pesticides

Pesticide	CAS No.	S_w (mg/L)[a]	log*P*	K_H (atm m^3/mol)[b]	Molecular weight (g/mol)	Maximum permissible level in soil (mg/kg)[c]
Atraton (ATN)	1610-17-9	180	2.69	$3.26 \cdot 10^{-9}$	211.26	N/A
Atrazine (ATRA)	139-40-2	35	2.61	$2.6 \cdot 10^{-9}$	215.68	0.01
Propazine (PROP)	333-41-5	8.6	2.93	$4.6 \cdot 10^{-9}$	229.71	0.05
Diazinon (DIAZ)	21087-64-9	60	3.81	$1.17 \cdot 10^{-7}$	304.35	0.1
Metribuzin (METR)	7287-19-6	10700	1.75	$1.2 \cdot 10^{-10}$	214.29	0.01
Prometryn (PROM)	7287-19-6	33	3.51	$1.32 \cdot 10^{-8}$	241.36	0.5
Oxyfluorfen (OXY)	42874-03-3	0.116	4.73	$1.2 \cdot 10^{-6}$	361.70	0.2

Note: [a]S_w – solubility in water at 20 °C; [b]K*H* – Henry's law constant at 25 °C; [c]Based on [17]

This study is aimed to develop a new method for the determination of organonitrogen pesticides in soil based on Vac-HSSPME and GC-MS. This is the first time the Vac-HSSPME approach is used for determination of pesticides in soil.

2. Experiment

2.1 Materials, reagents and instruments

Atraton (98%), atrazine (99%), propazine (98%), diazinon (98%), metribuzin (97%), prometryn (99%), oxyfluorfen (97%) were purchased from Sigma-Aldrich, (USA). High-purity acetonitrile (≥99.9%) and methanol (≥99.9%) purchased from Honeywell Riedel-de-Haën, (Germany) were used for preparation of stock solutions. Phosphate buffer pH 8.0 was used for adjusting the medium. Phosphate buffer was prepared by diluting 0.1308 g of potassium dihydrogen phosphate (99%, Reactiv, Russia) and 4.1825 g of potassium hydrogen phosphate (99%, Reactiv, Russia) in 250 mL of water. Extraction of pesticides was carried out in 20 mL crimp-top vials (HTA, Italy) sealed with modified Mininert® valve (Restek, USA) with fitted Thermogreen® LB-1 septum with half-hole (6×9 mm, Supelco, USA). Valves were prepared and modified as described in past [15, 16]. SPME fiber 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB, Supelco, USA) was used for the HS-SPME.

Laboratory vacuum pump 2VP-2 (Stegler, Russia) was used for air-evacuating the sample vial before headspace extraction. The magnetic stirrer PEX-6100 (Ecros, Russia) connected to the in-house made thermostat heating device with the temperature controller REX-C100 (Japan) and type K thermocouple 5TC-GG-K-20-36 (Omega, USA) were used for the extraction temperature control.

2.2 Parameters of gas chromatography–mass spectrometry analyses

GC-MS analyses were performed on the 6890N/5973N system (Agilent, USA). Desorption of analytes from PDMS/DVB fiber into GC inlet was conducted in spitless mode via 0.75 mm internal diameter inlet liner (Supelco, USA) at 240 °C for 10 min, with inlet purge activated at 5 min. Analytes were separated using a non-polar SLB-5MS column (30 m × 250 µm, 0.50 µm film thickness, Supelco, USA) at constant helium (≥99.995%, Orenburg-Tehgaz, Russia) flow 1.0 mL/min. Oven temperature was programmed from 100 °C (held for 5 min) to 200 °C with a heating rate 10 °C/min (held for 5 min), then to 300 °C with a heating rate 15 °C/min (held 1.33 min). Temperatures of interface, ion source and quadrupole MSD were 310, 230 and 150 °C, respectively. Mass spectrometric detection of analytes was performed in the selected ion monitoring (SIM) mode with electron impact ionization at 70 eV. The MS program used for the detection of target pesticides in the SIM mode is provided in Table 2. The first two ions with the highest intensity and mass were selected as quantifier and qualifier for each analyte based on respective mass spectrum.

2.3 Soil samples

Soil samples were collected in Almaty, Kazakhstan. The collected soil is a clay loam with organic matter content 0.9% determined using Gustavson's method. To remove possible residues and water, the soil was washed and dried in an oven for 2 h at 200 °C.

For preparation of model samples, the 2.00 g of soil were introduced into 20 mL crimp-top vials and spiked with 10 µL of working solution to provide a concentration of analytes at 200 ng/g. Vials were kept open for 15 min until evaporation of acetonitrile and were closed with a valve.

2.4 Vac-HSSPME procedure

The vials with soil samples were air-evacuated for 20 s. The air evacuation time was selected based on previous results for transformation products of unsymmetrical dimethylhydrazine [18]. The preset addition of distilled water was introduced using a gas-tight syringe (Bioject, China).

Table 2 – MS program for detection of analytes in SIM mode

Analyte	Retention time (min)	Quantifier ion, *m/z* (dwell 100 ms)	Qualifier ion, *m/z* (dwell 200 ms)	Group	Start time (min)
Atraton	19.17	211	196	1	18.50
Atrazine	19.71	200	215	2	19.35
Propazine	19.84	214	229		
Diazinon	20.52	179	304	3	20.00
Metribuzin	22.40	198	199		
Prometryn	22.80	241	226		
Oxyfluorfen	25.71	252	361	4	25.00

The samples were preincubated for 30 min at 60°C and 250 rpm, followed by an extraction step for the preset time. The SPME fiber was conditioned at 240 °C for 10 min under helium flow before each extraction. The valves and Thermogreen® septa were washed and dried at 100 °C for 1 h before use.

2.5 Ambient pressure HSSPME

For the ambient pressure HSSPME, 2.0 g of spiked soil sample and a preset water addition were introduced into a 20-mL crimp-top headspace vial, which was then sealed with PTFE/silicone septum and aluminum caps (Zhejiang Aijiren Technology Co., China). The sample was preincubated and extracted as described in section 2.4.

2.6 Study of the effect of water addition on responses of analytes

To the sealed vial with 2.0 g of soil sample, 0 or 3 mL of distilled water was added after air evacuation. The samples were pre-incubated and extracted for 30 min at 60°C.

2.7 Study of the effect of salting-out and pH adjustment on responses of analytes

For these experiments, 3.0 mL of water or phosphate buffer pH 8.0 and 0.90 g of sodium chloride were added to vials with soil prior to Vac-HSSPME. Air-evacuation was conducted after the salt addition step, prior to the buffer or water addition. Sample vials were preincubated and extracted for 30 min at 60 °C at constant stirring at 250 rpm.

2.8 Study of the effect of extraction temperature and time responses on analytes

Two Vac-HSSPME temperatures were studied: 30°C and 60°C. For obtaining extraction profiles of target pesticides, Vac-HSSPME was conducted for 5, 15, 30, 60 and 120 min at 60°C.

2.9 Method validation

Calibration curves were acquired in the concentration range of atraton 25 – 200 g/g, atrazine 25 – 200 ng/g, propazine 25 – 200 ng/g, diazinon 25 – 200 ng/g, metribuzin 6 – 55 ng/g, prometryn 6 – 55 ng/g and oxyfluorfen 25 – 200 ng/g. Five-point calibration curves were obtained at points 0, 6, 13, 40, 55 ng/g for metribuzin and prometryn, and points 0, 25, 50, 150, and 200 ng/g for other analytes.

Since the calibration data obtained using chromatographic methods are assumed to be heteroscedastic with the RSDs relatively constant in the entire calibration range [19, 20], weighted linear regression was used in this study with the weighting factor 1/x [20]. All calculations were conducted using Real Statistics package for MS Excel [21].

Intra-day (3 days) precision and inter-day precision were estimated as relative standard deviations of responses between respective triplicates. Recoveries were determined by analyzing model soil samples spiked with analytes at two concentration levels (75 and 100 ng/g) using optimized method. All experiments were conducted in triplicates.

3. Results and Discussion

3.1 Effect of pressure on HS-SSPME responses of analytes

When comparing the HS-SPME at ambient pressure and Vac-HSSPME, the substantial increase of responses was observed for all target pesticides (Figure 1). The positive effect of low pressure on HS-SPME performance is expected for

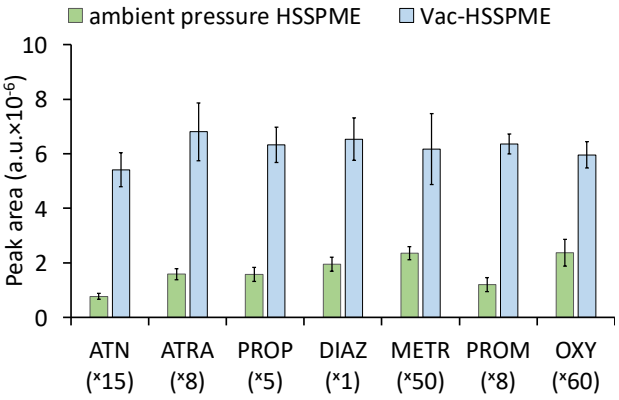

Figure 1 – Comparison of ambient pressure HS-SPME and Vac-HSSPME results

analytes with relatively low Henry's law constants and high fiber-air distribution constants [14]. In that case, the mass transfer of the compounds in the gaseous phase is the limiting step of the extraction process. Decreasing the pressure in the headspace results in accelerated diffusion of analytes in the gaseous phase. For the target pesticides, the 3-7 times increase in responses of analytes was observed at low pressure, demonstrating the good performance of the approach.

3.2 Effect of water addition

The addition of water to soil had substantial effect on responses of analytes (Figure 2). Addition of water to the soil is one of the methods to enhance extraction effectiveness and decrease soil's matrix effect during pesticides analysis [22, 23]. Water addition increases the sample's polarity, consequently enhancing the desorption kinetics from the sample to the headspace for nonpolar analytes [9]. This corresponds with the findings in this study. For analytes with the highest log*P* values, diazinon, prometryn and oxyfluorfen, the greatest increase in responses was observed after water addition, namely, by 380, 138 and 64 times, respectively (Figure 2). The responses of atraton were not detected without the addition of water. For other analytes, the 2-11 times increase in responses of analytes was observed. The lowest effect was observed for metribuzin, which can be explained by its highest solubility in water and the lowest Henry's law constant among target analytes, resulting in lower rates of mass transfer from the aqueous sample to the headspace.

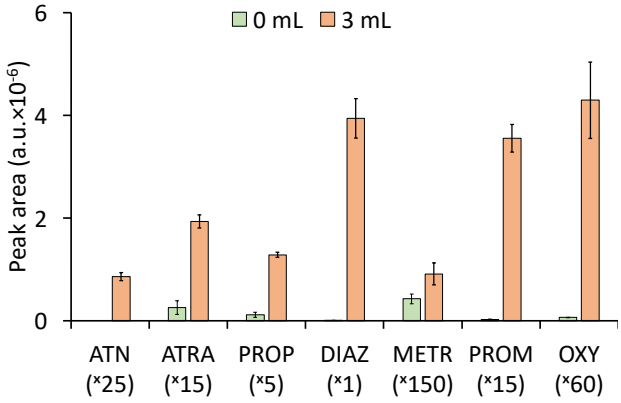

Figure 2 – Effect of water addition on responses of analytes obtained using Vac-HSSPME and GC-MS

3.3 Effect of salting-out and pH on responses of analytes

Salting-out is a common strategy for increasing the efficiency of HSSPME from aqueous or water-added samples [9, 24]. Since most target pesticides exhibit weak basic properties, the increase of sample pH was evaluated. Changing the pH to weakly alkaline medium decreases dissolution of basic pesticides in water, increasing the amount of pesticides in their neutral form that are available for extraction [25].

Positive effect of salting-out in combination with pH adjustment was observed for all analytes except for diazinon and oxyfluorfen (Figure 3). With salting-out and increase of pH (to pH 8.0), the increase of responses of triazines metribuzin (×6.5), atraton (×3.2), atrazine (×2.6), propazine (×2.5), and prometryn (×1.5) was observed compared to samples with neutral pH and no salt addition.

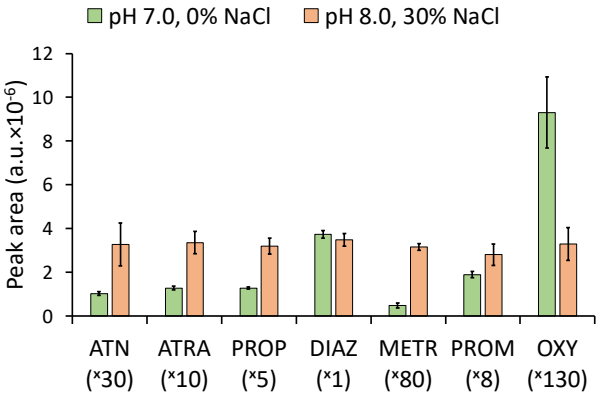

Figure 3 – Effect of salting-out and pH adjustment on Vac-HSSPME responses of analytes

Contrary, decrease in responses in the samples with salting-out and alkaline medium was observed for diazinon and oxyfluorfen. The negative effect of pH increase on responses for these analytes is explained by their chemical properties: oxyfluorfen is a diphenyl-ether, and diazinon is a thiophosphoric acid ester. The increase of sample pH thus favors dissociation of ester molecules resulting in lower fractions of analytes in the sample headspace. Considering that both have low dissociation constants in water, high *logP* values, and relatively high K_H values (Table 1), their amount in the sample headspace is still sufficiently high, allowing HSSPME even in alkaline media.

Thus, salting-out and increase of pH resulted in increased responses for most analytes. Therefore, the pH 8.0 and salting-out were chosen as optimum parameters for the following experiments.

3.4 Effect of extraction temperature and time on responses of analytes

The temperature increase in HSSPME has a negative effect on fiber-air distribution constants, and positive effect on mass transfer from sample to headspace. In case of pesticides' analysis in soil, which is the case of relatively low volatile analytes and highly binding matrix, the increase in extraction temperature generally results in higher extraction effectiveness [9], since limiting stage is the mass transfer from the sample to the headspace. At the same time, the positive effect of the low pressure on the diffusion of analytes in the gaseous phase decreases at elevated temperatures [18].

Vac-HSSPME-based responses of analytes were obtained at two different temperatures: 30°C and 60°C. Increase of the temperature to 60°C resulted in substantial increase in extraction effectiveness for all analytes (Figure 4). The greatest effect of temperature increase was observed for atraton (by 45 times), the lowest – for atrazine (by 8 times). Thus, mass transfer from the sample to headspace is the limiting process for the extraction of target analytes.

The Vac-HSSPME extraction profiles obtained for target pesticides in the range 5-120 min demonstrate that all analytes, except propazine, reached the equilibration time during 60 min extraction (Figure 5). For propazine the equilibrium was not achieved during 120 min of extraction. Generally, longer equilibration times for HSSPME are associated with high fiber-air distribution constants and low diffusion coefficients of analytes in fiber coating [26].

At the extraction time longer than 60 min, increase in standard deviations of responses was observed. Moreover, longer extraction times could be associated with negative effects on extraction effectiveness and decrease in the responses due to adsorption competition between analytes [15]. At extraction time 60 min the optimum combination of analytes responses and their standard deviations was achieved.

Thus, optimum Vac-HSSPME extraction parameters for target pesticides in soil are 60 min at 60 °C.

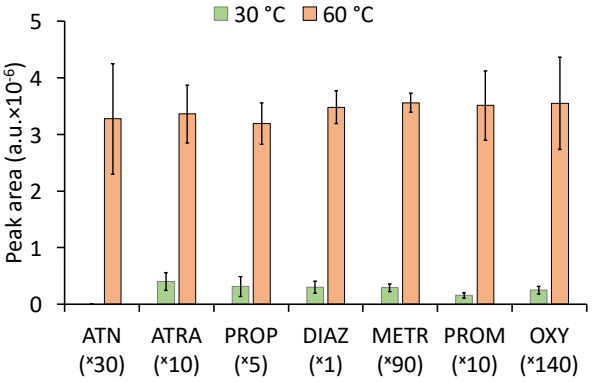

Figure 4 – Effect of extraction temperature on responses of analytes

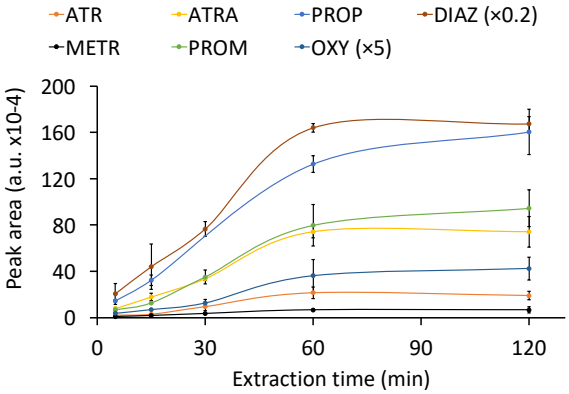

Figure 5 – Extraction profiles of pesticides

3.5 Analytical performance of the optimized method

The schematic workflow of the optimized method is provided in Figure 6.

HSSPME-based quantification is associated with matrix effects [27], therefore, matrix-matched calibration was used in this study to ensure better matrix effect control.

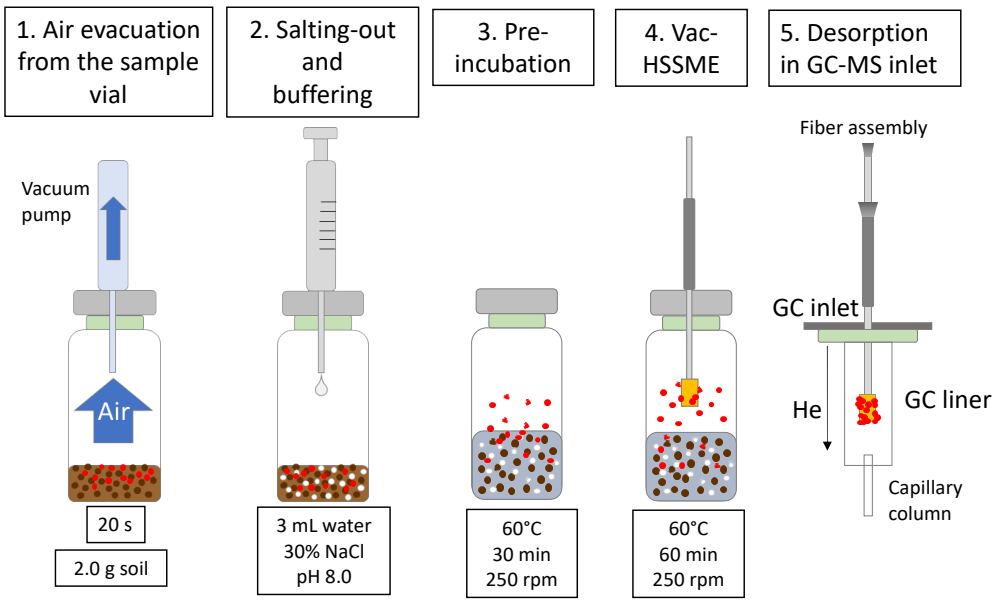

Figure 6 – Schematic representation of the proposed Vac-HSSPME-based method

Table 3 presents the linear ranges and linearity estimates for weighted curves, and limits of detection (LODs) and quantification (LOQs) for target pesticides obtained with the optimized method.

For most analytes, the weighted R^2 values were in the range 0.949-0.990. Poor linearity was observed for metribuzin, which can be attributed to its high water solubility (Table 1), which limits the performance of the headspace-based analysis from water-added samples. Therefore, the developed method cannot be recommended for metribuzin, and it was discarded from further results.

The spike recoveries determined using the optimized method were in the range 69-109% (Table 4).

Table 3 – Analytical performance of the optimized Vac-HSSPME method

Compound	Studied linear range (ng/g)	R^2	Slope	Inter-day precision (RSD, n=3)	Intra-day precision (RSD, n=3)	LOD (ng/g)	LOQ (ng/g)
Atraton	25 – 200	0.990	3541	20	10	4	12
Atrazine	25 – 200	0.995	1514	13	12	2	6
Propazine	25 – 200	0.989	5229	19	11	1	3
Diazinon	25 – 200	0.997	26577	18	18	0.1	0.4
Metribuzin	6 – 55	0.726	1452	25	25	NA	NA
Prometryn	6 – 55	0.964	23846	14	10	0.3	1
Oxyfluorfen	25 – 200	0.949	1228	22	14	1	2

Note: The LODs and LOQs were calculated as concentrations that provide 3 and 10 signal-to-noise (S/N) ratios, respectively. S/N were measured in calibration standards with lowest concentration of each analyte.

Table 4 – Spike recoveries using the developed method based on Vac-HSSPME

Analyte	Concentration level 1			Concentration level 2		
	Spiked (ng/g)	Measured (ng/g)	Recovery (%)	Spiked (ng/g)	Measured (ng/g)	Recovery (%)
Atraton	76	54	71±10	102	70	69±7
Atrazine	78	55	70±22	104	114	109±16
Propazine	77	56	73±22	102	93	91±16
Diazinon	77	73	95±18	103	78	76±21
Prometryn	19	15	77±24	25	19	77±16
Oxyfluorfen	76	66	87±18	101	73	73±26

3.6 Comparison of the developed method with other known methods

The developed Vac-HSSPME-based method was compared with other known methods for the determination of some target pesticides in soil (Table 5). Both GC and LC-based methods are used for target pesticides. QuEChERS is used for multiresidue sample preparation prior to either GC or LC-based analysis [28, 29]. Majority of the sample preparation methods use solvent extraction (solid-liquid extraction, SLE) followed by sample clean-up steps such as solid-phase extraction (SPE), dispersive liquid-liquid microextraction (DLLME) [30], or SPME [31, 32]. The sample preparation based on preliminary solvent extraction of soil are indispensably complicated by additional steps of filtration and centrifugation, in addition to required clean-up. Compared to these methods, the Vac-HSSPME-based method proposed in this study offers faster and simpler single-step and solvent-free extraction while achieving similar or better detection limits.

The proposed method exhibits similar detection limits and duration of sample preparation with other SPME-based method [33], in which fiber coating based on polymeric ionic liquid was used for extraction of analytes at elevated extraction temperatures (90 °C). The use of Vac-HSSPME allows to achieve higher extraction efficiencies at lower temperatures, which might be critical for thermolabile compounds.

Table 5 – Comparison of the developed method with other known methods

Analyte(s)	Sample preparation	Analytical istrument	LOD (ng g^{-1})	Recovery (%)	Refe-rence
Triazines (7 pesticides)	SLE (dichloromethane) + MAE for 30 min at 50 °C) + SPME (30 min)	HPLC-UV	NA	81-106	[31]
Triazines (atrazine, simazine, terbumeton, terbuthylazine, terbutryn)	SLE (methanol), MAE + SPME (30 min)	GC-MS	3	82	[32]
Triazines (11 pesticides)	SWE (water/ethanol) + SPE	HPLC-PDA	0.4 – 3.3	79 – 101	[34]
Triazines (propazine, simazine, atrazine)	SLE (UAE) + SPE	HPLC-UV	3.32 – 4.82 nmol/kg	78 – 100	[35]
Atrazine	SLE (UAE) + MSPE + DLLME	GC-NPD	0.05	95 – 103	[30]
218 pesticide and metabolites, including triazines	QuEChERS	LC-MS/MS, GC-MS/MS	0.024-6.25	70 – 120	[28]
Oxyfluorfen (and metazachlor, q uizalofop-p-ethyl, quinmerac, α(±)-cypermethrin)	QuEChERS	GC-MS	0.45	70.8-105.7	[29]
Oxyfluorfen	HS-SPME (60 min at 90 °C)	GC-MS	0.1	95-101	[33]
Triazines (atraton, atrazine, prometryn, propazine), oxyfluorfen, diazinone	Vac-HSSPME (60 min at 60 °C)	GC-MS	0.1-3.6	69-109	This study

Note: SLE – solid-liquid extraction; SWE – supercritical water extraction; SPE – solid-phase extraction; MAE – microwave-assisted extraction; MSPE – magnetic solid-phase extraction; HPLC-UV – high-performance liquid chromatography-ultraviolet, PDA – photodiode array detector; NPD – nitrogen–phosphorus detector

4. Conclusion

In this study, the vacuum-assisted HSSPME approach was applied for the first time for quantification of pesticides in soil.

Enhanced extraction effectiveness of Vac-HSSPME compared to ambient pressure HSSPME at the same extraction time was demonstrated for target pesticides. Therefore, the Vac-HSSPME is a promising approach for solvent-free extraction of other semi-volatile pesticides, which are otherwise difficult to extract using headspace-based sampling methods.

The optimized Vac-HSSPME-based method involves salting-out and pH adjustment to pH 8.0 followed by extraction for 60 min at 60°C. The proposed method offers green, fast and simple alternative for quantification of target pesticides in soil while providing similar or better detection limits. Compared to previously reported SPME-based method in analytes extracted at elevated temperature (90 °C) [33], the proposed Vac-HSSPME method offers similar sensitivities at milder extraction conditions, which are more suitable for thermolabile compounds.

Detection limits of the proposed method are at least an order of magnitude lower than the maximum permissible levels for the target pesticides in soil. Thus, the method can also be used at shorter extraction times for higher throughput, while providing acceptable sensitivity of measurements.

The proposed method can be recommended for quantification of atraton, atrazine, propazine, diazinon, prometryn, and oxyfluorfen in soil. However, poor analytical performance of the method was observed for metribuzin, which can be explained by its high water solubility, and thus, limited mass transfer from the water-added sample to the headspace. Therefore, the method cannot be used for quantification of metribuzin in soil.

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