Determination of 1-methyl-*1H*-1,2,4-triazole in soils contaminated by rocket fuel using solid-phase microextraction and isotope dilution gas chromatography – mass spectrometry

Saltanat Yegemova[1], Nadezhda V. Bakaikina[1], Bulat Kenessov[1*], Jacek A. Koziel[2], Mikhail Nauryzbayev[1]

[1]al-Farabi Kazakh National University, Center of Physical Chemical Methods of Research and Analysis, Almaty, Kazakhstan, s.egemova@gmail.com; bakaikina@cfhma.kz; bkenesov@cfhma.kz

[2] Iowa State University, Department of Agricultural and Biosystems Engineering, Ames, IA, USA; koziel@iastate.edu

* - Corresponding author, 050012 Almaty, 96a Tole bi Street, lab. 101, Kazakhstan, tel: +7 727 2921374; fax: +7 727 2923731; e-mail: bkenesov@cfhma.kz.

Abstract

Environmental monitoring of Central Kazakhstan territories where heavy space booster rockets land requires fast, efficient, and inexpensive analytical methods. The goal of this study was to develop a method for quantitation of the most stable transformation product of rocket fuel, i.e., highly toxic unsymmetrical dimethylhydrazine – 1-methyl-1H-1,2,4-triazole (MTA) in soils using solid-phase microextraction (SPME) in combination with gas chromatography – mass spectrometry. Quantitation of organic compounds in soil samples is complicated by a matrix effect. Thus, an isotope dilution method was chosen using deuterated analyte (MTA-d3) for matrix effect

control. The work included study of the matrix effect, optimization of sample equilibration stage (time and temperature) after spiking MTA-d3 and validation of the developed method. Soils of different type and water content caused an order of magnitude difference in SPME effectiveness of the analyte. Isotope dilution minimized matrix effects. However, proper equilibration of MTA-d3 in soil was required. Complete MTA-d3 equilibration at temperatures below 40°C was not observed. Increase of temperature to 60°C and 80°C enhanced equilibration reaching theoretical MTA/MTA-d3 response ratios after 13 and 3 h, respectively. Recoveries of MTA depended on concentrations of spiked MTA-d3 during method validation. Lowest spiked MTA-d3 concentration (0.24 mg kg^{-1}) provided best MTA recoveries (99-121%). Addition of excess water to soil sample prior to SPME increased equilibration rate, but it also decreased method sensitivity. Method detection limit depended on soil type, water content, and was always below 1 mg kg^{-1}. The newly developed method is fully automated, and requires much lower time, labor and financial resources compared to known methods.

Keywords: solid-phase microextraction; 1-methyl-1H-1,2,4-triazole; soil; soil equilibration; internal standard; matrix effect.

1. Introduction

1.1 Toxicity of rocket fuel and its transformation products

Contamination of soil by highly toxic rocket fuel unsymmetrical dimethylhydrazine (UDMH) and its transformation products causes many health risks, especially for human population and ecosystem of territories close to take-off routes of heavy rockets “Proton”, “Dnepr” and “Cyclone” launched from Baikonur cosmodrome [1]. Most toxic UDMH transformation products are N-nitrosodimethylamine, dimethylamine, tetramethyltetrazene and 1-methyl-*1H*-1,2,4-triazole (MTA) [2]. Among all transformation products, MTA is most stable and has highest

concentrations in soils of fall out places reaching 100 mg kg^{-1} [3]. According to the proposed mechanism, formaldehyde dimethylhydrazone is the main intermediate during a two-step formation of MTA from UDMH [4]. Experimentally determined lethal dose for MTA at its intravenous, intraperitoneal and peroral administrations are 510±20, 750±40 and 1020±60 mg kg^{-1} body weight, respectively [5,6]. Long-term MTA administration leads to changes in lymph and hemodynamics, cell and protein composition of blood, and affects the state of cell membranes. Recently, the maximum allowable concentration of MTA in soil 10 mg kg^{-1} was recommended for introduction in Kazakhstan [7].

1.2 Current methods for quantitation of MTA in environmental samples

Methods for determination of MTA in environmental samples are based on gas (GC) and liquid chromatography (LC) in combination with various detectors (Table 1). Most popular methods are based on organic solvent extraction followed by evaporative concentration and analysis on GC with mass spectrometric (MS) detector [8–10]. These methods are quite labor/time consuming and require toxic organic solvents. When polar organic solvents are used, water present in soil is also extracted and injected to GC negatively affecting column and MS filament lifetime. Methods based on LC [10–12] have high efficiency for analysis of water and methanol extracts, but require quite expensive MS detector for proper selectivity and sensitivity. LC-MS instruments are not available in laboratories of Kazakhstan responsible for environmental impact assessment of Baikonur cosmodrome. Monitoring of rocket landing locations requires analysis of many soil samples. Cost of single analysis significantly affects the total budget of monitoring, number of samples that can be analyzed and reliability of monitoring studies.

Table 1. Methods used for determination of MTA in soils.

Analytes	Sample preparation	Method	MTA detection limit(s)	Reference
Volatile transformation products of UDMH	Extraction by acetone : methylene chloride (1:1) in ultrasound	GC-MS	60 mg L^{-1} (in extract)	[8]
MTA	Extraction by acetone or methylene chloride	GC-MS	0.005 mg kg^{-1}	[9]
MTA, FDMH	Soxhlet extraction by methanol	GC-MS	0.02 mg kg^{-1}	[10]
Transformation products of UDMH	Extraction by 0.1 M HCl during 24 h with periodic shaking, centrifugation	LC-MS/MS	0.006 µg L^{-1} (in extract)	[11]
MTA	Extraction by water	LC-DAD	0.2 mg L^{-1} (in extract)	[12]
Transformation products of UDMH	Extraction by ammonia solution (pH 10), centrifugation and filtration	LC-MS	0.5 mg L^{-1} (in extract)	[13]
Volatile transformation products of UDMH	Headspace SPME by 85 µm Car/PDMS, extraction time 60 min, temperature 40°C	GC-MS	Screening, n/a	[14]

MTA	Injection of 10 µL of MTA-d3 (24 mg L^{-1}). Equilibration during 5 h at 80ºC, headspace SPME by 85 µm Car/PDMS, extraction time 1 min, temperature 80°C	GC-MS	Depends on soil type, worst case: 1 mg kg^{-1}	This study

Note: GC - gas chromatography; MS – mass spectrometry; LC - high performance liquid chromatography; DAD - diode-array detection; SPME - solid phase microextraction; HS - headspace; UDMH - unsymmetrical dimethylhydrazine; MTA - 1-methyl-*1H*-1,2,4-triazole; MTA-d3 - 1-(trideuteromethyl)-*1H*-1,2,4-triazole ; FDMH - 1-formyl-2,2-dimethylhydrazine; Car – Carboxen; PDMS – polydimethylsiloxane; n/a – not available.

Recently a new method based on headspace solid-phase microextraction (SPME) and GC-MS was developed for screening of UDMH transformation products in soil samples [14]. The method is based on extraction of analytes by a thin polymer coating from headspace of soil or soil-water mixture in a closed vial followed by thermal desorption in a heated GC injection port (Fig. 1). It combines extraction, cleanup and concentration in a single step, does not require organic solvents and allows quick, inexpensive and fully automated analysis of many soil samples [3]. SPME is especially robust when extracting analytes from headspace, because only volatile compounds reach fiber coating without physical contact with matrix, thus significantly increasing its lifetime. Compared to solvent extraction, SPME does not lead to injection of non-volatile compounds and impurities (such as soil particles) to GC injection port and minimizes its maintenance.

In spite of numerous advantages of SPME, quantitative determination of organic contaminants in soil is complicated due to matrix effect on extraction effectiveness of analytes [15]. The same problem exists also for other solid samples including food [16], food packaging [17] construction materials [18], and solid waste [19]. SPME is a non-exhaustive extraction method. For soil samples, SPME effectiveness depends on the equilibrium between headspace and sample (Fig. 1). Increase of water content in soil leads to the decrease of peak areas of UDMH transformation products, due to their high polarity and affinity to water [14]. Other important parameters affecting SPME effectiveness are mechanical composition [20] and organic carbon content [19,21]. Currently, there are no methods for quantitative determination of MTA in soils based on SPME.

1.3. Strategies for minimizing soil matrix effects

Several methods were proposed to minimize matrix effect:

- addition of excess water [21–26];
- preliminary extraction by organic solvent followed by its evaporation and addition of excess water [27,28];
- exhaustive extraction by cold SPME fiber [29,30];
- standard addition [22]; and
- internal standard [19,21,31].

Figure 1

Addition of excess water leads to the increase of detection limits for polar analytes [14,30] and inaccurate results due to formation of complexes [19]. Pre-SPME extraction by organic solvent and cold fiber SPME significantly increase complexity of analysis and lead to additional expenses.

For matrix effect control, standard addition [22] and internal standard [19,21,31] methods are used. However, after introduction of standard to soil sample, they must be equilibrated before SPME. Llompart et al. [22] added excess water and used standard addition method for matrix effect compensation during determination of *o*-, *m*-, *p*-xylenes and 1,2-, 1,3-, 1,4-dichlorobenzenes. Samples were equilibrated by shaking for 30 min and storing for 24 h prior to analysis. Authors reported that the proposed method could not be used for analysis of semivolatile organic compounds due to their slow kinetics of distribution in soil at room temperature.

A method for determination of phenol and 3-chlorophenol in soil based on internal standard was described by Baciocchi et al. [21]. To compensate for matrix effect, excess water was added followed by direct immersion SPME. Parameters of sample equilibration after introduction of standard were not reported. Wang et al. [31] developed a method for determination of 16 priority polycyclic aromatic hydrocarbons (PAHs) in soil. Before analysis, internal standard containing three isotopically-labeled PAHs was introduced to samples followed by 2 h shaking and 24 h storage in darkness. Then, samples were extracted by hexane:methylene chloride mixture under ultrasound followed by centrifugation, solvent evaporation, addition of water and direct immersion SPME. Results presented by Higashikawa et al. [19] show that the internal standard method does not allow for effective control of the matrix effect because physicochemical properties of solid samples differently affect SPME efficiency of VOCs. For equilibration after internal standard addition, solid samples were kept at 20°C for 1 h. Smirnov et al. [10] reported efficient equilibration of MTA added to soil by dissolving it in a large volume of dichloromethane followed by a solvent evaporation. However, this method requires toxic organic solvent and increases costs of analysis.

1.4. Identification of knowledge gaps

In spite of the fact that a soil equilibration after a standard addition can affect the results of analysis, available publications did not focus on its impact and optimization. Equilibration during

24 h used by many authors significantly affects overall speed of analysis. Addition of excess water may enhance equilibration process, but negatively affects method sensitivity.

1.5. Objectives

The goal of the present study was to develop a method for quantitative determination of MTA in soil samples by SPME-GC-MS involving isotope dilution. The work included (a) study of the matrix effect on SPME effectiveness, (b) optimization of sample equilibration stage after introduction of internal standard, (c) determination of the optimal concentration of internal standard and (d) validation of the developed method by analysis of real soil samples with known analyte concentrations.

2. Experimental

2.1 Materials and reagents

1-Methyl-*1H*-1,2,4-triazole (purity >99%) and 1-(trideuteromethyl)-*1H*-1,2,4-triazole (purity 98.3%) were synthesized by Dr. Nurzhan Kurmankulov and Dr. Kseniya Bortnikova from A.B. Bekturov Institute of Chemical Sciences using methods described in articles [32,33]. Synthesis of 1-(trideuteromethyl)-*1H*-1,2,4-triazole (MTA-d3) was done using iodomethane-d3 (Sigma-Aldrich, USA). Purities of both substances were determined by direct injection GC-MS. According to mass spectrum collected during elution of the overlapped MTA and MTA-d3 peak, concentration of MTA in synthesized MTA-d3 was 1.7±0.2% (Fig. S1).

HPLC grade methanol (AppliChem, Germany) and chemically pure methylene chloride (ECOS-1, Russia) were used in this work as organic solvents.

2.2 Instrumentation

Experiments were conducted using two GC-MS systems: 6890N/5975C and 7890A/5975C TAD (both – Agilent, USA) equipped with HT280T (HTA, Italy) and Combi-PAL (CTC

Analytics, Switzerland) SPME autosamplers, respectively. Separation was done on a 30 m × 0.25 mm, 0.25µm film HP-Innowax (Agilent, USA) columns at constant flow of helium (>99.995%, Orenburg-Tehgas, Russia) equal to 1 mL min^{-1}. Oven temperature of 6890N GC was programmed from 40ºC (held for 10 min) to 100ºC (held for 10 min) at a 10ºC/min ramp followed by a 10ºC/min ramp to 200ºC. For faster analysis, oven temperature of 7890A GC was programmed from 40ºC (held for 1 min) to 100ºC (held for 11 min) at a 10ºC/min ramp followed by a 20ºC/min ramp to 200ºC. Detection was done in selected ion monitoring (SIM) mode. Molecular ions of MTA and MTA-d3 having m/z 83 and 86 amu, respectively, were simultaneously detected using dwell times 50 ms. Temperatures of MS interface, ion source and quadrupole were 240, 230 and 150°C, respectively. Solvent delay was set to 12 min.

SPME was conducted using autosamplers. For HT280T autosampler, 65 µm PDMS/DVB fiber (Supelco, USA) was used. Combi-PAL autosampler was equipped with a more efficient 85 µm Car/PDMS (Supelco, USA) [14]. All experiments were conducted using 20 mL clear crimp-top vials and PTFE/Silicone septa (HTA, Italy) pre-conditioned at 150ºC for 2 h.

2.3 Preparation of reference soil samples

Experiments for method development were conducted on reference soil samples taken from landing regions of first stages of rocket carriers located in Central Kazakhstan. Sand, light, medium and heavy loam had concentrations of humus equal to 0.12, 0.60, 0.33, 0.90% and pH 7.0, 8.4, 8.5 and 8.8, respectively. For method validation, soil having concentration of humus 13.2% was also used. Soils with such high concentration of humus do not exist in rocket landing areas in Central Kazakhstan, but many landing areas (e.g., in Eastern Kazakhstan and Altay, Russia) may have such soils. Soils were passed via 1 mm sieve and dried at 150°C during 4 h for preparation of reference samples with known concentration of MTA and water content.

2.3.1 Studies of matrix effect

For study of matrix effect, four types of soils with MTA concentrations 10 mg kg^{-1} and water content 5, 10, 15, 20, 25 and 30% were prepared. 300, 250, 200, 150, 100 and 50 µL of MTA solution in distilled water with concentrations 33.3, 40, 50, 66.7, 100 and 200 mg L^{-1}, respectively, were added to 20 mL vials with 1.00 g of dried soils. Immediately after injection, samples were crimped, shaken for 15 min and stored in darkness for 1 week before analysis. All samples were prepared in duplicates. Total of 48 vials were prepared.

2.3.2 Effects of temperature and time on equilibration

170 g of light and medium loam were placed into two 250 mL flasks for experiments to optimize temperature and time of spiked soil equilibration. Then, 4.00 mL of MTA solution (C=424 mg L^{-1}) in methanol and 100 mL of methylene chloride were consequently added. After spiking, soil samples were left under working fume hood for 2 d to evaporate solvents. When using such method, losses of MTA are possible. To determine concentration of MTA in prepared samples, they were analyzed according to [10] using Soxhlet extraction by methanol during 8 h. For higher accuracy, before extraction, 2.00 g of soil was spiked with 10 µL of MTA-d3 solution in methanol (C = 540 µg mL^{-1}). Concentration was determined by multiplication of C (MTA-d3) by the ratio of MTA and MTA-d3 peak areas. Determined concentrations of MTA in light and medium loam were 7.2 mg kg^{-1} being equivalent of 72% analyte recovery.

2.4 Methodology of experiments

2.4.1 Study of a matrix effect on SPME effectiveness of MTA from soil sample headspace

Prepared soil samples of different type and water content (section *2.3.1*) were extracted by 65 µm PDMS/DVB fiber at 50ºC during 5 min. Analysis was completed on an Agilent 6890N/5975C equipped with HT280T autosampler. Pre-incubation time was 10 min, desorption was done in GC inlet heated to 240ºC during 5 min. After analysis, 5 mL of distilled water was added to soil samples having water content of 5, 15 and 30%. After re-crimping of vials, they were intensively

shaken for 15 min, held for 12 h at room temperature and analyzed again. After analysis, MTA-d3 was added to every vial followed by intensive agitation for 15 min, 12 h storage at room temperature and another analysis. Samples with water content 10 and 20% were spiked with 23 µL of MTA-d3 solution in water (C=444 µg mL^{-1}) followed by intensive shaking for 15 min, 2 d storage at room temperature and analysis. After analysis, 5 mL of 1 M HCl was added to every vial, vials were shaken, kept for 7 d and analyzed.

2.5 Optimization of temperature and time of soil equilibration after spiking MTA-d3

Three 20 mL vials each containing 1.00 g of prepared (section *2.3.2*) medium loam were spiked with 10 µL of MTA-d3 solution in water (C = 1200 mg L^{-1}) at the interval of 30 min, sealed, placed in the agitator of Combi-PAL autosampler, and heated to desired temperature (30, 40, 60, 80 or 120^{0}C). SPME was performed immediately after spiking and every 90 min till the complete stabilization of MTA/MTA-d3 response ratio (i.e., apparent equilibration). SPME was conducted by 85 µm Car/PDMS fiber during 1 min at the studied equilibration temperature. Desorption in GC injector was conducted for 2 min at 240 °C. Analyses were conducted on an Agilent 7890A/5975C TAD.

2.6 Study of MTA-d3 equilibration after spiking into soils of different type and water content.

Selected water volume (0, 100, 250 or 400 µL) was added to 20 mL vials containing 1.00 g of selected soil sample (light or medium loam, section *2.3.2*) followed by intensive shaking for 5 min. Three replicates were prepared for every soil type and water content. Every vial was spiked with 10 µL MTA-d3 water solution, immediately sealed and placed in Combi-PAL agitator heated to 60°C. SPME was started immediately after spiking and performed every 90 min till complete stabilization of MTA/MTA-d3 response ratio. Three replicate samples having the same soil type and water content were analyzed consecutively. SPME was conducted by 85 µm Car/PDMS fiber

during 1 min at 60°C. Desorption was conducted during 2 min at 240 °C. Analyses were conducted on an Agilent 7890A/5975C TAD.

2.7 Method validation

Validation of the developed method was performed on soils (section *2.3.2*) of a different type and water content with known MTA concentrations: light loam (20%, 10.0 mg kg^{-1} and 0%, 1.00 mg kg^{-1}), medium loam (40%, 0.50 mg kg^{-1}), sand (10%, 5.00 mg kg^{-1}) and soil having high humus content (water content 30%, 1.00 mg kg^{-1}). Vials containing 1.00 g of soil sample were spiked with 10 µL of MTA-d3 solution (C = 24 mg L^{-1}), intensively shaken for 5 min, kept in the Combi-PAL agitator or a drying oven at 80°C for 5 h and extracted with 85 µm Car/PDMS fiber for 1 min at 80°C. Analyses were conducted on Agilent 7890A/5975C TAD. Fibers were conditioned for 5 min before and after extraction in the rear GC inlet equipped with a straight 1.5 mm liner and heated to 310°C for increased analysis accuracy, to lower analyte carryover, and to minimize interferences from lab air. Inlet flow rate was set to 15 mL min^{-1}.

After analyses were completed, vials with samples were decapped, spiked with 10 µL of MTA-d3 solution in water (C=120 mg L^{-1}), again equilibrated at 80°C for 5 h and analyzed using the same parameters. After these analyses, vials with samples were decapped again, spiked with 10 µL of MTA-d3 solution in water (C=1200 mg L^{-1}), equilibrated again at 80°C for 5 h and analyzed. After analyses, 5.00 mL of distilled water was added to every vial, resulting slurries were shaken in the agitator at 500 rpm for 100 min and analyzed.

3. Results and Discussion

3.1 Effect of soil type and water content

Soil type and water content significantly affect MTA extractions with SPME (Fig. 2a). Increase of water content in sand from 5 to 30% led to an ~11-fold decrease of MS detector

response to MTA. For light and medium loam, ~2-fold decrease was observed. Maximum extraction effectiveness of MTA from sand & light loam and heavy loam was obtained at 5% and 20% water content, respectively. Highest affinity to MTA was observed for heavy loam, probably, due its higher specific surface area. Most efficient extraction of MTA was achieved from sand having larger particles and lowest surface area. Maximum MTA response (sand, 5% water) was ~15 times higher than the lowest one (light loam, 25% water). This indicates that results obtained by SPME and external standard calibration only, may be as variable as ~1 order of magnitude lower or higher than the real value. Thus, external standard calibrations for MTA are not recommended for soil analyses.

Addition of excess water slightly decreases matrix effect (Fig. 2b). The maximum observed response of MTA was ~8 times higher compared to the lowest one. Thus, accurate determination of MTA in soil by SPME requires effective matrix control. Internal standard method was selected involving isotopically labeled (deuterated) MTA-d3 having molecular mass 86 amu and relatively inexpensive reagents for synthesis. As generally known, isotopically labeled internal standard is ideal because it has same physicochemical properties as the analyte.

Figure 2.

3.2 Matrix effect control with internal standard

Internal standard method involving MTA-d3 was very efficient in establishing apparent equilibrium for sand and reaching MTA/MTA-d3 response ratio very close to theoretical (1.0) (Fig. 3a). For loamy soils, water content had a very significant effect on how well the soil was equilibrated. It could be caused by a complexity of MTA-d3 equilibration after its injection into soil sample. Addition of acid to soil samples spiked with MTA-d3 improved the resulting MTA/MTA-d3 response ratio closer to theoretical. However, differences still reached 50% (light loam) and 20-30% standard deviations (SDs) (Fig. 3b).

Figure 3.

The use of MTA-d3 internal standard addition for quantitation of MTA by SPME was thus proven to be feasible. However, it became apparent that parameters of soil equilibration after spiking with MTA-d3 need to be optimized. Injected MTA and water are likely bound to limited number of soil particles located on the soil sample surface near the bottom of the vial. Intensive shaking allows more even distribution of MTA in water and soil volume only for soils having lower water content (typically <10%). However, complete equilibration requires equal distribution of MTA-d3 molecules over all soil particles as the equilibrated analyte itself. Soils having higher water content may form clumps, size of which cannot be always decreased by shaking. This would make equilibration process more complex and longer.

3.3. Role of diffusion and adsorption on equilibration of MTA-soil

Molecules of volatile organic compounds in soil diffuse via gas or liquid (water) phases. Therefore, rate of MTA diffusion depends on its concentration in gas and liquid phases. Taking into account complex chemical composition of soil, it might be useful to introduce a concept of "weak", "medium" and "strong" adsorption sites having different affinity to MTA (Fig. 5). Most analyte molecules already equilibrated in soil are probably adsorbed on sites to which they have high affinity (signified by yellow cross and the 'strong' adsorbent route). After spiking with internal standard, MTA-d3 will be adsorbed on all remaining sites. If MTA and MTA-d3 concentration is the same, ratio of their concentrations among three types of adsorbents will not be equal to 1.0 because MTA molecules were initially adsorbed onto "strong" sites. Uniform equilibration of both analyte and internal standard will require additional time depending on MTA affinity to soil and its concentration in gas phase. Lowest method accuracy will be observed if MTA is irreversibly adsorbed on some ('strong' adsorbent type) soil particles (Fig. 4). MTA-d3 molecules will not readily substitute MTA molecules and complete equilibration among various

types of adsorbents will not be achieved. Adsorption effectiveness at such sites may be decreased by the increase of temperature and/or water content in soil.

However, as was shown (Fig. 2a), increase of water content in soil is undesirable and leads to decrease of SPME effectiveness on MTA and overall method sensitivity. Increase of temperature is preferred because it also leads to the increase of MTA concentration in headspace and SPME effectiveness. The goal of the next experiment was to study and optimize temperature for quick and effective equilibration of MTA-d3 after its injection to soil.

Figure 4.

3.4 Optimization of temperature and time of soil equilibration after spiking MTA-d3

Equilibration of MTA-d3 progressed very slowly at 30°C (Fig. 5). Complete equilibration was not observed even 22 h after injection of MTA-d3 standard. At 40°C , MTA/MTA-d3 response ratio was stabilized 18 h after spiking. Increase of temperature to 60 and 80°C decreased equilibration time to 13 and 3 h, respectively. At 120°C, equilibration was reached immediately after spiking, most probably due to the presence of most MTA in the gas phase.

Equilibration at T>60°C provided very close (±4%) MTA/MTA-d3 responses ratios. At these temperatures, ratio of MTA/MTA-d3 concentrations (0.60) was equal to ratios of their responses at equilibrium. Ratios of responses were 14 and 26% lower than expected at 40 and 30°C, respectively. This may indicate that complete equilibration at these temperatures was not reached. At 40°C, MTA/MTA-d3 responses ratio was already stabilized (i.e., not changing with time anymore) meaning that complete equilibrium may never be practically reached at such temperature (Fig. 5) confirming the proposed concept (Fig. 4).

Figure 5.

Standard deviations at 30 and 40°C reached 21% and were much higher compared to 7% at 60 and 80°C. It may be caused by different distribution of non-equilibrium adsorption sites in different soil samples and time dependent desorption. It negatively affects effectiveness of matrix control by internal standard method. Therefore, MTA-d3 equilibration temperature must be at least 60°C during at least 12 h. When rapid sample preparation and analysis is required, e.g., in field conditions, 120°C may be used, but special care must be taken to avoid explosion of vials when analyzing samples having water content >10%. Optimal temperature for general field monitoring purposes is 80°C.

3.5 Equilibration of MTA-d3 in soils of different type and water content

As shown earlier (Figs. 2 and 3), soils have different chemical composition and affinity to MTA which may also affect the *rate* of soil equilibration after spiking internal standard. Increase of water content may accelerate equilibration due to a faster diffusion via water film covering soil particles. However, wet soils may form clumps and slow down equilibrium. The goal of this experiment was to establish MTA-d3 equilibration time in soils of different type and water content.

MTA-d3 equilibrated in 6-10 h after spiking light and medium loam at all studied water contents. Increase of water content enhanced equilibration (Fig. 6). After equilibration, MTA/MTA-d3 responses ratio were in the range of ±5% variability indicating high precision and efficiency of the method of isotopically labeled internal standard for controlling effect of soil type and water content.

Figure 6.

3.6 Validation of the developed method

Analysis of reference soil samples with known concentrations of MTA showed good accuracy of the developed method, particularly at lowest concentrations of MTA-d3 (0.24 mg kg-

^{1}) (Table 2). At this concentration, recovery was between 91 and 121% for all studied soils. Increase of MTA-d3 concentration led to a decrease of the method accuracy; recovery was 96-147%. Highest deviation from real MTA concentration was observed for soil having the lowest content of analyte (0.5 mg kg^{-1}). It may be caused by proximity to detection limit for this soil being 0.5 mg kg^{-1}. At this concentration, chromatographic matrix peaks partially overlaid MTA peak and affected its peak area. MTA response may also be higher than expected because of the input of the MTA-d3 solution also containing MTA. This hypothesis was confirmed by the increase of MTA recovery to 181% with further increase of MTA-d3 concentration to 13.4 mg kg^{-1}. Such MTA-d3 concentration contains 0.25 mg kg^{-1} MTA and causes the increase of recovery by ~50%. Recoveries of MTA from soils having highest analyte concentrations were 99-127% and were much less affected by the additional input of MTA with added solution of MTA-d3.

Table 2. Method validation with reference soils having known concentrations of MTA.

Soil type	Water content (%)	Reference MTA (mg kg^{-1})	Measured concentration of MTA (mg kg^{-1})				Recovery (%)			
			Concentration of added MTA-d3 (mg kg^{-1})							
			0.24	1.44	13.4	13.4 (+5 mL H_2O)	0.24	1.44	13.44	13.4 (+5 mL H_2O)
Medium loam	40	0.5	0.6 ±0.1	0.70 ±0.05	0.9 ±0.1	0.92 ±0.04	116 ±16	147 ±9	181 ±16	185 ±8
Light loam	1	1	1.20 ±0.03	1.10 ±0.03	1.20 ±0.01	1.30 ±0.03	121 ±3	109 ±3	123 ±1	128 ±3
Soil w/ high humus content	30	1	1.0 ±0.2	1.1 ±0.1	1.30 ±0.02	1.10 ±0.04	99 ±17	115 ±10	127 ±2	115 ±4
Sand	10	5	4.5 ±0.2	4.8 ±0.1	4.9 ±0.1	4.5 ±0.1	91 ±4	96 ±3	99 ±1	92 ±2

Light loam	20	10	9.6 ±0.3	10.1 ±0.2	10.3 ±0.9	10.0 ±0.1	96 ±3	101 ±2	103 ±9	100 ±1

Results indicate that MTA-d3 must be added at lowest feasible concentrations. Higher concentrations of MTA-d3 result in poorer accuracy and higher consumption of expensive isotopically-labeled internal standard. Analysis of a single soil sample will require 0.24 µg of MTA-d3 to reach target 0.24 mg kg^{-1} concentration of added MTA-d3. At such consumption plus triplicate losses, 1 g of MTA-d3 will be enough for more than 1 M analyses. In addition, detection limit of MTA-d3 is about 20 times lower compared to MTA due to a lower noise and the absence of matrix peaks at m/z 86 SIM chromatogram (Fig. S2). Thus, it guarantees high precision at concentration of MTA-d3 0.24 mg kg^{-1}. Addition of excess water (5 mL) to 1 g of analyzed soils did not affect method accuracy. As expected, it led to significant decrease of MTA response and overall method sensitivity. Thus, addition of excess water to analyzed sample is not recommended.

3.7 Estimation of method detection limit

MTA detection limit was different for different soil type samples because MTA extraction efficiency varied by an order of magnitude (Fig. 3a). In this case, method detection limit can be established for a worst-case scenario represented by the soil with highest surface area and highest water content (Fig. S2). For such soils, MTA detection limit was 1.0 mg kg^{-1}, being an order of magnitude lower than its recommended maximum allowable concentration in soils from rocket landing regions [7].

In spite of the fact that this method has lower sensitivity compared to many methods described in the literature (Table 1), it has many other advantages including shorter time, labor and materials expenses. The developed method does not require toxic organic solvents, may be fully automated and even used in the field together with portable GC-MS.

4. Conclusions

A new method for quantitative determination of 1-methyl-*1H*-1,2,4-triazole in soils based on solid-phase microextraction and GC-MS was developed. Effectiveness of MTA extraction from soils of different type and water content may vary by an order of magnitude. For matrix effect control, internal standard method was proposed involving deuterated MTA (MTA-d3, MW=86). It was shown that the increase of the rate and the efficiency of MTA-d3 equilibration may be achieved by the increase of temperature. Minimal recommended temperature for equilibration of MTA-d3 was 60°C. For equilibration at such temperature, 10 h are required. At 30 and 40°C, soil samples could not be completely equilibrated, probably to the presence of strong MTA adsorption sites. Increase of equilibration temperature to 80°C allows complete equilibration during 5 h. For immediate equilibration of dry soils, temperature may be increased to 120°C. Increase of water content also resulted in the increase of equilibration rate, but it also decreased overall method sensitivity. The developed method was successfully validated on soil samples having different type, water content and MTA concentration. MTA recovery depended on spiked concentration of MTA-d3, particularly for lowest MTA concentrations. Optimal concentration of spiked MTA-d3 was 0.24 mg kg^{-1} providing recoveries ranging from 99-121%. Addition of excess water did not result in better method accuracy. Method detection limit depended on soil type, water content, but could not be higher than 1 mg kg^{-1}. Compared to known methods, the developed method is fully automated and requires much lower time, labor and financial resources.

5. Acknowledgements

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 Saltanat Yegemova both funded by the Ministry of Education and Science of Kazakhstan.

Authors are grateful to Dr. Nurzhan Kurmankulov and Dr. Kseniya Bortnikova (Institute of Chemical Sciences, Almaty, Kazakhstan) for synthesis of 1-methyl-*1H*-1,2,4-triazole and 1-methyl-*1H*-1,2,4-triazole-d3.

Authors would like to extend sincere thanks to Dr. Michael L. Thompson, Department of Agronomy, Iowa State University, for his constructive feedback on importance of soil physicochemical properties and practical considerations for soil sampling and analyses.

References*:*

[1] L. Carlsen, B.N. Kenessov, S.Y. Batyrbekova, A QSAR/QSTR study on the environmental health impact by the rocket fuel 1,1-dimethyl hydrazine and its transformation products., Environ. Health Insights. 1 (2008) 11–20.

[2] L. Carlsen, B.N. Kenessov, S.Y. Batyrbekova, A QSAR/QSTR study on the human health impact of the rocket fuel 1,1-dimethyl hydrazine and its transformation products. Multicriteria hazard ranking based on partial order methodologies, Environ. Toxicol. Pharmacol. 27 (2009) 415–423. doi:10.1016/j.etap.2009.01.005.

[3] B. Kenessov, M. Alimzhanova, Y. Sailaukhanuly, N. Baimatova, M. Abilev, S. Batyrbekova, et al., Transformation products of 1,1-dimethylhydrazine and their distribution in soils of fall places of rocket carriers in Central Kazakhstan, Sci. Total Environ. 427-428 (2012) 78–85. doi:10.1016/j.scitotenv.2012.04.017.

[4] B. Kenessov, L. Carlsen, Identification (quantitative determination and detection) and fate of transformation products of rocket fuel 1,1-dimethylhydrazine, in: Transform. Prod. Emerg. Contam. Environ., 2014: pp. 627–648. doi:10.1002/9781118339558.ch21.

[5] S. Batyrbekova, System Analysis of Environment Objects of Territories of Kazakhstan, Negatively Affected by the Activity of the “Baikonur” Cosmodrome, Diss. Dr. Chem. Sci. Degree. (2010).

[6] M. Nauryzbayev, Report on the project “Creation of a system of physical chemical diagnostics of concentrations of rocket fuel components in environmental objects,” 2006.

[7] Z. Zhubatov, V.A. Kozlovskiy, Report on the project “Evaluation of stability of natural ecosystems affected by space rocket activity. Experimental studies on justification of maximum allowable concentrations of toxic rocket fuel components and their transformation products,” 2012.

[8] A.K. Buryak, O.G. Tataurova, A.V. Ulyanov, Study of transformation products of unsymmetrical dimethylhydrazine on model sorbents by the method of gas chromatography/mass spectrometry, Russ. J. Mass Spectrom. (2004) 147–152.

[9] B. Kenessov, S. Batyrbekova, M. Nauryzbayev, T. Bekbassov, M. Alimzhanova, L. Carlsen, GC-MS determination of 1-methyl-1H-1,2,4-triazole in soils affected by rocket fuel spills in Central Kazakhstan, Chromatographia. 67 (2008) 421–424. doi:10.1365/s10337-008-0535-4.

[10] R.S. Smirnov, I.A. Rodin, A.D. Smolenkov, O.A. Shpigun, Determination of the products of the transformation of unsymmetrical dimethylhydrazine in soils using chromatography/mass spectrometry, J. Anal. Chem. 65 (2010) 1266–1272. doi:10.1134/S1061934810120117.

[11] D.S. Kosyakov, N.V. Ul’yanovskii, K.G. Bogolitsyn, O.A. Shpigun, Simultaneous determination of 1,1-dimethylhydrazine and products of its oxidative transformations by liquid chromatography–tandem mass spectrometry, Int. J. Environ. Anal. Chem. 94 (2014) 1254–1263. doi:10.1080/03067319.2014.940342.

[12] B.N. Kenessov, Y. Sailaukhanuly, B. Musrepov, A. Kaldarov, HPLC determination of 1-methyl-1H-1,2,4-triazole in water, Chem. Bull. Kazakh Natl. Univ. (2008) 184–190.

[13] I.A. Rodin, I.A. Anan'eva, A.D. Smolenkov, O.A. Shpigun, Determination of the products of the oxidative transformation of unsymmetrical dimethylhydrazine in soils by liquid chromatography/mass spectrometry, J. Anal. Chem. 65 (2010) 1405–1410. doi:10.1134/S1061934810130150.

[14] B.N. Kenessov, J.A. Koziel, T. Grotenhuis, L. Carlsen, Screening of transformation products in soils contaminated with unsymmetrical dimethylhydrazine using headspace SPME and GC-MS, Anal. Chim. Acta. 674 (2010) 32–39. doi:10.1016/j.aca.2010.05.040.

[15] E. Boyacı, Á. Rodríguez-Lafuente, K. Gorynski, F. Mirnaghi, É.A. Souza-Silva, D. Hein, et al., Sample preparation with solid phase microextraction and exhaustive extraction approaches: Comparison for challenging cases, Anal. Chim. Acta. (2014). doi:10.1016/j.aca.2014.12.051.

[16] A.A. Rincón, V. Pino, J.H. Ayala, A.M. Afonso, Multiple headspace solid-phase microextraction for quantifying volatile free fatty acids in cheeses, Talanta. 129 (2014) 183–190. doi:10.1016/j.talanta.2014.05.032.

[17] A.A. Argyri, A. Mallouchos, E.Z. Panagou, The dynamics of the HS/SPME-GC/MS as a tool to assess the spoilage of minced beef stored under different packaging and temperature conditions, Int. J. Food Microbiol. 193 (2015) 51–58. doi:10.1016/j.ijfoodmicro.2014.09.020.

[18] S.J. Sun, J. Shen, Z.Y. Zhao, Study on determination method of the volatile organic compounds emissions from particleboards for indoor finishing, Adv. Mater. Res. 250-253 (2011) 935–938. doi:10.4028/www.scientific.net/AMR.250-253.935.

[19] F.S. Higashikawa, M.L. Cayuela, A. Roig, C.A. Silva, M.A. Sánchez-Monedero, Matrix effect on the performance of headspace solid phase microextraction method for the analysis of target volatile organic compounds (VOCs) in environmental samples, Chemosphere. 93 (2013) 2311–2318. doi:10.1016/j.chemosphere.2013.08.023.

[20] R.D. Durovic, J.S.G. Umiljendic, S.B. Cupac, L.M. Ignjatovic, Solid phase microextraction as an efficient method for characterization of the interaction of pesticides with different soil types, J. Braz. Chem. Soc. 21 (2010) 985–994.

[21] R. Baciocchi, M. Attinà, G. Lombardi, M.R. Boni, Fast determination of phenols in contaminated soils, J. Chromatogr. A. 911 (2001) 135–141.

[22] M. Llompart, K. Li, M. Fingas, Headspace solid phase microextraction (HSSPME) for the determination of volatile and semivolatile pollutants in soils, Talanta. 48 (1999) 451–459.

[23] A. Soler, M. Lebrun, Y. Labrousse, T. Woignier, Solid-phase microextraction and gas chromatography-mass spectrometry for quantitative determination of chlordecone in water, plant and soil samples, Fruits. 69 (2014) 325–339. doi:10.1051/fruits/2014021.

[24] A. Peruga, J. Beltrán, F. López, F. Hernández, Determination of methylisothiocyanate in soil and water by HS-SPME followed by GC–MS–MS with a triple quadrupole, Anal. Bioanal. Chem. 406 (2014) 5271–5282. doi:10.1007/s00216-014-7960-z.

[25] K. Farhadi, S. Bochani, M. Hatami, R. Molaei, H. Pirkharrati, Gas chromatographic detection of some nitro explosive compounds in soil samples after solid-phase microextraction with carbon ceramic copper nanoparticle fibers, J. Sep. Sci. 37 (2014) 1578–1584. doi:10.1002/jssc.201400144.

[26] M. Alimzhanova, B.N. Kenessov, M. Nauryzbayev, J.A. Koziel, Effects of moisture content and solvent additive on headspace solid-phase microextraction of total petroleum hydrocarbons from soil, Eurasian Chem. J. 14 (2012) 331–335.

[27] J. Beltran, F.J. López, F. Hernández, Solid-phase microextraction in pesticide residue analysis, J. Chromatogr. A. 885 (2000) 389–404.

[28] R.D. Durović, T.M. Dorđević, L.R. Santrić, S.M. Gasić, L.M. Ignjatović, Headspace solid phase microextraction method for determination of triazine and organophosphorus pesticides in soil, J. Environ. Sci. Health. B. 45 (2010) 626–632. doi:10.1080/03601234.2010.502416.

[29] J. Guo, R. Jiang, J. Pawliszyn, Determination of polycyclic aromatic hydrocarbons in solid matrices using automated cold fiber headspace solid phase microextraction technique, J. Chromatogr. A. 1307 (2013) 66–72. doi:10.1016/j.chroma.2013.07.110.

[30] R. Jiang, E. Carasek, S. Risticevic, E. Cudjoe, J. Warren, J. Pawliszyn, Evaluation of a completely automated cold fiber device using compounds with varying volatility and polarity, Anal. Chim. Acta. 742 (2012) 22–29. doi:10.1016/j.aca.2012.01.010.

[31] Y. Wang, J. Zhang, Y. Ding, J. Zhou, L. Ni, C. Sun, Quantitative determination of 16 polycyclic aromatic hydrocarbons in soil samples using solid-phase microextraction, J. Sep. Sci. 32 (2009) 3951–3957. doi:10.1002/jssc.200900420.

[32] M.R. Atkinson, J.B. Polya, Triazoles. Part II. N-substitution of some 1 : 2 : 4-triazoles, J. Chem. Soc. (1954) 141. doi:10.1039/jr9540000141.

[33] 1,2,4-TRIAZOLE, Org. Synth. 40 (1960) 99. doi:10.15227/orgsyn.040.0099.

Figure captions

Fig. 1. Solid-phase microextraction of organic compounds from soil. Stages of SPME: 1 – fiber assembly (before extraction); 2 – extraction; 3 – thermal desorption; C_F – analyte concentration in fiber; C_{HS} – analyte concentration in headspace; C_{Soil} – analyte concentration in soil; C_W – analyte concentration in water.

Fig 2. Mass spectrum of synthetized MTA-d3 used as internal standard for development of the method

Fig. 3. Effect of soil type and water content on peak area of MTA by headspace SPME-GC-MS. Note: A – no additives; B – 5 mL of water added; mass of dry soil = 1.00 g; 65 µm PDMS/DVB fiber; extraction time = 5 min; extraction temperature = 50 ºC.

Fig. 4. Effect of soil type and water content (10 and 20%) on MTA/MTA-d3 responses ratio by headspace SPME-GC-MS. Notes: A – no additives; B – 5 mL of 1M HCl added. Conditions: dry soil weight = 1.00 g; extraction time = 10 min; extraction temperature = 50ºC; 65 µm PDMS/DVB fiber.

Fig. 5. Proposed mechanism of incomplete equilibration of MTA and MTA-d3 in soil sample after spiking MTA-d3.

Fig. 6. Dependence of MTA/MTA-d3 response ratio on time at temperatures = 30, 40, 60, 80 and 120°C. Conditions: light loam; water content = 0%; extraction time = 1 min; 85 µm Car/PDMS fiber.

Fig. 7. Equilibration of MTA-d3 after its injection to soils having different type and water content at 60ºC. Conditions: A – light loam; B – medium loam; studied water contents: 0, 10, 25, 40%; extraction time = 1 min; 85 µm Car/PDMS fiber.

Fig. 8. Chromatogram obtained by SPME-GC-MS of light loam having water content 1%, MTA (m/z 83 amu) concentration = 1 mg kg^{-1} and MTA-d3 (m/z 86 amu) concentration = 0.24 mg kg^{-1}.