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ARTICLES

Highly Sensitive Determination of 1,1-Dimethylhydrazine by High-Performance Liquid Chromatography–Tandem Mass Spectrometry with Precolumn Derivatization by Phenylglyoxal

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Abstract—A new highly sensitive and rapid approach to the determination of 1,1-dimethylhydrazine in natural water is developed (determination range is 0.03–1 μg/L). It is based on the use of high-performance liquid chromatography–tandem mass spectrometry with precolumn derivatization by phenylglyoxal and does not require any preconcentration. Derivatization, chromatographic separation conditions, and tandem mass spectrometry detection parameters are chosen. Intra-day precision of the results of measurements of 1,1-dimethylhydrazine in natural water is 12–16%, and inter-day precision is 16–22%. The lowest limit of detection and the lowest limit of quantification are 0.010 μg/L and 0.030 μg/L, respectively.

Keywords: 1,1-dimethylhydrazine, tandem liquid chromatography–mass spectrometry, high performance liquid chromatography, precolumn derivatization, phenylglyoxal

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INTRODUCTION

1,1-Dimethylhydrazine (unsymmetrical dimethylhydrazine, UDMH) finds widespread use in rocket and space activities as a fuel for heavy launch vehicles. It is a highly toxic compound, which belongs to the 1st hazard class and has carcinogenic, embryotoxic, gonadotoxic, and allergenic effects and polytropic action, and causes poisoning in all ways of intake [1]. In Russia, the following health-based exposure limits were established: UDMH threshold limit value (TLV) in soil is 0.1 mg/kg [2] and in water reservoirs for agricultural use it is 0.5 μg/L [3], and UDMH tentative permissible level (TPL) in water of water reservoirs for household use is 0.06 μg/L [4].

Several approaches to the chromatographic determination of UDMH have been proposed previously. Ion chromatography with amperometric detection ensures the determination of UDMH in the native form [5], and determination by HPLC and gas chromatography requires precolumn derivatization [6]. Aromatic aldehydes are the most widely used derivatization agents [7–9]; the use of glyoxal along with HPLC and solid phase extraction was reported [10, 11]. In addition, approaches to the determination of UDMH by ion chromatography [12], and reversed-phase (RP) HPLC with mass spectrometric detection [13] were described. However, all these methods either do not have sufficient sensitivity, or require laborious sample preparation (including sample preconcentration stage). The most promising way to increasing the sensitivity of UDMH determination is a combination of RP HPLC with tandem mass spectrometric detection and precolumn derivatization. The choice of the derivatization agent should be based on the formation of a stable product with a rather high molecular weight (to reduce background noise) and the presence of functional groups, contributing to ionization under the conditions of electrospray ionization or atmospheric pressure chemical ionization at, in the structure of the forming derivative. As was shown in [14], glyoxals are promising reagents for the determination of UDMH because of mild derivatization conditions and the presence of a system of conjugated bonds in the structure of the derivative with UDMH, which ensures the improvement of analytical characteristics both in spectrophotometric detection and in the use of MS/MS detection in combination with ESI in the positive mode. However, the low molecular weight of the used reagents prevents the achievement of a gain in the sensitivity of MS/MS detection as compared with the spectrophotometric detection. In this study, phenylglyoxal was proposed for derivatization, because its derivative with UDMH has good characteristics both for the use of RP HPLC and for mass spectrometric detection.

The aim of this study was to develop a rapid procedure for the highly sensitive determination of UDMH in waters at TLV and TPL without the preconcentration of the sample.

Operation parameters of the electrospray ionization source

Gas temperature of peripheral layer, T °C	400
Gas flow of peripheral layer, L/min	12
Drying gas temperature, T °C	350
Drying gas flow, L/min	11
Sprayer pressure, atm	3.4
Capillary voltage, V	2500
Needle voltage, V	0

EXPERIMENTAL

Solutions and reagents. The following reagents were used in the study: 1,1-dimethylhydrazine, phenylglyoxal monohydrate, caffeine (all Sigma-Aldrich, United States), ammonium acetate, acetic acid, acetonitrile (for gradient HPLC) (all Panreac, Spain), and deionized water (Milli-Q, Millipore, United States).

Equipment. Chromatographic analyzes were carried out on liquid chromatographs Agilent 1200 (Agilent Technologies, United States), consisting of a four-channel gradient pump, an auto-injector with a possibility of thermostatting the samples, a column thermostat, and a diode-array detector and Agilent 1290 (Agilent Technologies, United States), consisting of a two-channel gradient pump, an automatic injector with a possibility of thermostatting the samples, a column thermostat, and a diode-array detector, and an Agilent 6460 tandem triple quadrupole mass spectrometer (Agilent Technologies, United States) equipped with a source of atmospheric pressure chemical ionization (APCI) and electrospray ionization with Agilent Jet Spray technology (ESI). To increase the volume of the injected sample, an Agilent Large Volume Injection Kit was used.

Data collection and the processing of chromatograms were carried out using the MassHunter Workstation software B 06.00.00 software package(Agilent Technologies, United States) and Agilent Chemstation software package (Agilent Technologies, United States).

Chromatographic separation conditions. The separation of the sample components was performed in the isocratic mode on Zorbax SB-C18 columns (150 × 4.6 mm, particle size 3.5 μm, and pore size 80 Å, Agilent technologies, United States) and Zorbax SB-C18 (150 × 4.6 mm, particle size 5 μm, and pore size 80 Å, Agilent technologies, United States) with 3 × 4 mm Security Guard precolumn with the C18 phase (Phenomenex, United States). The volume of the injected sample was 100 μL. Separation was carried out the in isocratic mode. The composition of the mobile phase was 65% of a 20 mM ammonium acetate buffer solution (pH 5.4) and 35% of acetonitrile. Flow rate was 1 mL/min.

The choice of derivatization conditions was performed by studying the dependences of the areas of chromatographic peak of the UDMH derivative with phenylglyoxal on different parameters in diode-array detection at the wavelength λ_{det} = 340 nm.

Sample preparation. Derivatization was carried out directly in the day of the experiment. One milliliter of a 1,1-dimethylhydrazine solution with a prescribed concentration or a test sample, 20 μL of a 1 M ammonium acetate buffer with pH 5.0, and 25 μL of the reagent (2% phenylglyoxal solution) were introduced into a sealed plastic tube. The reaction was carried out for 10 min at 70°C. Then, 20 μL of a caffeine solution (internal standard) with the concentration 1 mg/L was introduced into 1 mL of the reaction mixture cooled to room temperature; the mixture was stirred and injected into the chromatograph.

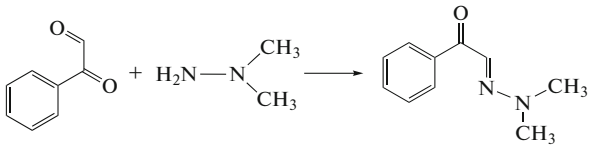
Operation parameters of the ionization source. An electrospray ionization source with the Agilent JetSpray technology was used in the positive ion mode. The operation parameters of the ionization source are presented in the table.

Conditions of tandem mass spectrometric detection. Measurements were carried out in the mode of selected reaction monitoring by m/z 177 → 105 (quantitative analysis), m/z 177 → 77 (confirmation transition) for the phenylglyoxal derivative of UDMH, and m/z 195 → 138 for caffeine (internal standard) ion transitions. Collision energies were 10, 34, and 20 eV, respectively. Declustering potential was 80 and 100 V for the phenylglyoxal derivative of UDMH and caffeine, respectively, and scan time was 300 ms.

Calculations of UDMH content of the sample were carried out using the internal standard method: the analytical signal was the ratio of peak areas the UDMH derivative of phenylglyoxal (m/z 177 → 105 ion transition) to the peak area of the internal standard.

RESULTS AND DISCUSSION

Selection of derivatization conditions. The presence of two carbonyl groups in the phenylglyoxal molecule may favor the formation of some condensation products with UDMH. However, because of the higher activity of the aldehyde group under the conditions of excess reagent, the reaction proceeded with the formation of a single product:



To carry out the reaction at room temperature in a 500-fold molar excess of the reagent, more than 1 h was needed; to increase the rapidity of the approach, it was proposed to carry out the reaction at an elevated

temperature. From the dependences of the area of the chromatographic peak of the product of UDMH reaction with phenylglyoxal, it was found that, at 50, 60, and 70°C, the reaction proceeded for 20, 15, and 10 min, respectively. At higher temperatures, the yield of the derivative decreased; therefore, to achieve the maximum sensitivity and rapidity of the analysis, it was advisable to carry out the reaction at 70°C. The mechanism of the condensation of aldehydes with hydrazines includes the stage of general acid catalysis; at the same time, in highly acidic media, the protonation of hydrazines is possible. Therefore, the reaction yield should depend on the pH of the reaction medium. We studied the pH dependence of the area of the chromatographic peak of the UDMH derivative, which had a maximum in the pH range 3.5–5.5. The pH of the reaction medium was adjusted by adding phosphate or ammonium-acetate buffer solutions with the necessary pH value to the concentration 20 mM. As the use of phosphate buffer solutions was undesirable in using the HPLC/MS/MS method, in the further work, to adjust the pH of the reaction medium to the desired value, we used an ammonium acetate buffer solution with pH 5.0–5.5.

The choice of derivatization conditions was performed at the maximum possible phenylglyoxal concentration corresponding to a saturated solution. However, it was shown that a 4-fold decrease in the reagent concentration (0.05% of phenylglyoxal in the reaction mixture) did not result in a decrease in the reaction yield. This allowed us to reduce the cost out of the analysis and the interfering effect of impurities in the reagent.

The chosen derivatization conditions were as follows: 20 mM ammonium acetate buffer solution with pH 5.0–5.5; heating to 70°C for 10 min; reagent concentration in the reaction mixture 0.05%.

Selection of conditions of chromatographic separation. To obtain data necessary for the selection of derivatization conditions, we used a Zorbax SB-C18 reversed-phase column (150 × 4.6 mm, particle size 5 μm, and pore size 80 Å, Agilent technologies, United States), which previously showed good results in the separation of hydrophobic UDMH derivatives [14]. The mobile phase was a 20 mM ammonium acetate buffer solution with pH 5.4 to which acetonitrile was added. The buffer solution was needed for the conversion of the UDMH derivative into an uncharged form and the stabilization of retention parameters. When more than 30% of acetonitrile was added to the mobile phase and spectrophotometric detection (used in choosing derivatization conditions) was used, the separation of the peak of the UDMH derivative with phenylglyoxal could not been separated from the peaks of impurities; therefore, separation was performed at a 25% acetonitrile concentration in the mobile phase. With the transition to highly selective MS/MS detection, the concentration of acetonitrile in the mobile phase was increased to 35%. In combination with a Zorbax SB-C18 column (150 × 4.6 mm, particle size 3.5 μm, and pore size 80 Å, Agilent technologies, United States), this allowed us to increase the efficiency and, correspondingly, sensitivity of determination.

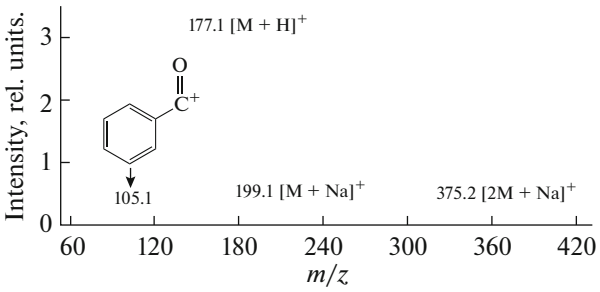


Fig. 1. Mass spectra of product of UDMH interaction with phenylglyoxal. Ionization mode: ESI(+), c_{UDMH} = 1 μg.

Selection of MS/MS detection conditions. To achieve the maximum sensitivity and selectivity of analysis, it was necessary to carry out detection in the mode of selected reaction monitoring after optimization of the parameters of detection and sample ionization.

Selection of the mode and ionization parameters. The presence of two nitrogen atoms in the molecule of the phenylglyoxal derivative of UDMH provides the maximum ionization yield in the positive ion mode. In this case signal intensity using APCI was two orders of magnitude lower than that obtained using electrospray ionization. To increase the sensitivity of the determination, operation parameters of the electrospray ionization source were chosen. The choice of the parameters was carried out by comparing the areas of chromatographic peaks of the derivative by varying the operation parameters of the ESI source in the Source Optimizer program of the Agilent MassHunter Workstation software package. The chosen parameters are shown in the table.

Selection of ion transitions. To choose a precursor ion, we recorded a chromatogram of the reaction mixture of UDMH with phenylglyoxal by the total ion current and studied the mass spectrum of the derivative (Fig. 1). The most intensive signal in the mass spectrum (m/z 177) corresponds to the protonated molecule of the phenylglyoxal derivative with UDMH, which was chosen as the precursor ion.

The selection of product ions, declustering potential, and collision energy was performed in an automatic mode using the Optimizer program of Agilent MassHunter Workstation software package, which ensures the reduction of the time of selection of the parameters by varying them within recording one chromatogram. Because of an increase in noise in recording masses with low m/z values, chromatograms by the total ion current of product ions were obtained

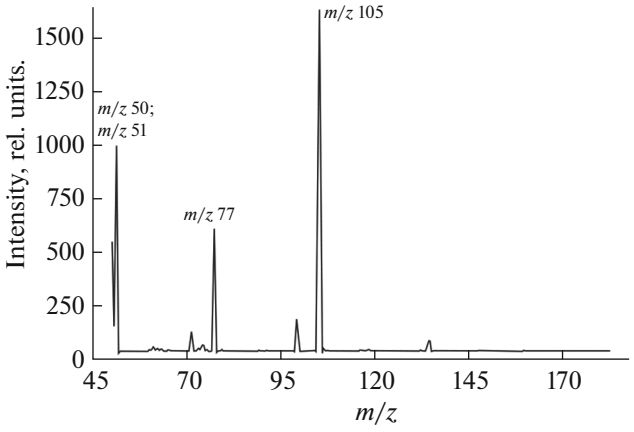


Fig. 2. A MS/MS spectrum of an UDMH derivative with phenylglyoxal. c_{UDMH} = 0.5 μg.

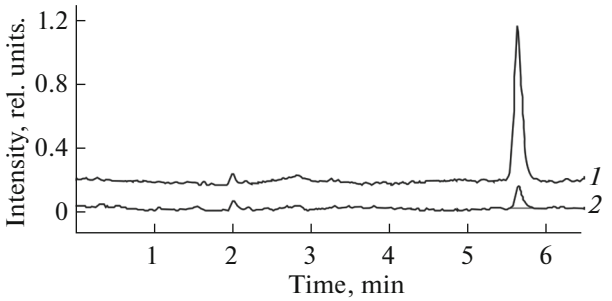


Fig. 3. Overlapping chromatograms of (*1*) phenylglyoxal/UDMH reaction mixture in the mode of selected reaction monitoring by the m/z 177 → 105 ion transition. c_{UDMH} = 0.03 μg/L, and (*2*) phenylglyoxal solution. $t_{reaction}$ = 10 min, T = 70°C, pH 5.0, $c_{phenylglyoxal}$ = 0.05%. $V_{injected\ sample}$ = 100 μL.

in the range m/z 50–190. A spectrum of product ions is shown in Fig. 2. The ion with m/z = 105 with the maximum intensity in the spectrum was chosen for quantitative analysis; to increase the reliability of analysis, ion with m/z = 77 was chosen and as a confirmation ion, because it has rather high molecular weight and its signal is highly intense. The dependences of the intensities of signals of corresponding product ions on collision energy were studied; it was found that the intensities have maxima at 10 eV (m/z = 105) and 34 eV (m/z = 77), which corresponds to the maximum yield of ions in the dissociation of the precursor ion in the collision cell.

Selection of the internal standard. The efficiency of ionization in using ESI may vary within wide limits, e.g., because of different degrees of source pollution; therefore, to increase the reproducibility of analysis, an internal standard was added to the sample. Because of the absence of commercially available compounds similar to the detected hydrazone in structure, in this study caffeine was proposed as the internal standard. Caffeine does not interact with the analytes and is stable, and nitrogen atoms present in its structure are responsible for its ability to ionize in the ESI(+) mode. The determination of Caffeine by HPLC/MS/MS was widely discussed in the literature [15]. It was detected by the m/z 195 → m/z 138 ion transition. Caffeine was added to the sample in the concentration up to 20 μg/L.

Determination of performance characteristics and tests on real objects. To estimate the linearity range of the method, the dependence of chromatographic responses on UDMH concentration was studied. To take into account possible deviations in the operation of the detector instead of concentration dependences of the areas of chromatographic peaks of the detected compounds, we constructed concentration dependences of the ratio of peak areas to the peak area of the internal standard. As one can see in Fig. 3, there is a small peak of the derivative in the chromatogram of the blank sample, which, apparently, is due to the pollution of the reagent with trace amounts of UDMH. When constructing a calibration curve, a peak of the blank sample was taken into account by subtracting the chromatogram of the blank sample from the chromatograms of calibration solutions. The obtained calibration curve (n = 3) was linear in the concentration range 0.03–1 μg/L.

The quantification limit was determined as the lowest value of the linearity range. The limit of detection was calculated as UDMH concentration in the sample, at which the signal-to-noise ratio was equal to 3. The limit of detection and the quantification limit were 0.01 and 0.03 μg/L; therefore, the proposed version of the procedure ensures the determination of UDMH in waters at a level of 1/2 TPL without preconcentration.

The proposed procedure for UDMH determination was tested by analyzing various samples of natural water containing 0.06 and 0.5 μg/L of UDMH (TPL and TLV levels, respectively). A sample of lake water selected at the territory of the Altai Republic, in clean parts of areas allocated for the intake of second stages of "Proton" carrier rockets, a sample of tap water, and a sample from a well initially containing no ecotoxicants were used as test samples. Validation was carried out by the added–found method, convergence was estimated w by three results of UDMH determination in samples carried out in one day, and intermediate precision was estimated by the results obtained within 5 days. The reproducibility and repeatability were 16–22% (n = 5) and 12–16% (n = 3), respectively. As one can see from the data obtained, the proposed approach is characterized by acceptable accuracy, reproducibility, and precision.

Figure 4 shows overlapping chromatograms of a sample of natural water with an addition of 0.06 μg/L of UDMH, of a standard UDMH solution, and of the blank sample. One can see from the chromatograms

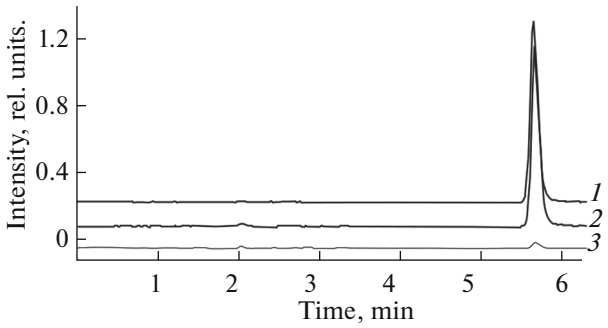


Fig. 4. Overlapping chromatograms of (*1*) reference solution with c_{UDMH} = 0.06 μg/L, (*2*) sample of natural water with an addition of 0.06 μg/L of UDMH, and (*3*) sample of natural water with no UDMH additive after derivatization by phenylglyoxal in the mode of selected reaction monitoring by the m/z 177 → 105 ion transition. $t_{reaction}$ = 10 min, T = 70°C, pH 5.0, $c_{phenylglyoxal}$ = 0.05%. $V_{injected\ sample}$ = 100 μL.

that, in the analysis of natural samples in the selected reaction monitoring mode, peaks of impurity components of the matrix were not present in the chromatograms, which ensures analyses without additional sample pretreatment.

CONCLUSIONS

The use of phenylglyoxal for derivatization in the determination of UDMH by HPLC/MS/MS was proposed. The conditions of derivatization and chromatography–mass spectrometrometry analysis were chosen. The high selectivity and sensitivity of the approach ensure the reliable determination of UDMH in water samples with the minimum sample pretreatment at the levels of TPL and TLV without sample preconcentration.

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