Determination of 7 nitrobenzene compounds in soil, glass, and cotton cloth by ultrahigh performance liquid chromatography

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Abstract. An ultrahigh performance liquid chromatographic (UHPLC) method has been developed for detection of 7 nitrobenzene compounds (nitrobenzene, 2-nitrotoluene, 1, 3, 5-trinitrotoluene, 3-nitrotoluene, 3, 4-dinitrotoluene, 2, 6-dinitrotoluene, 2, 4, 6-trinitrotoluene) in soil, glass and cotton cloth. Analytes were extracted twice by acetonitrile. 200 µL of the extract solution was pipetted to a tube with 800 µL water. After filtration by a nylon membrane, the analytes were determined by UHPLC with a phenyl modified column. The linear correlation coefficients of more than 0.99 were achieved. The detection range was examined from 0.05 to 5.0 mg/L. In this method, the limit of detection (LOD) of 0.5 mg/kg was obtained. Recoveries in spiked samples were more than 87.8%, and the relative standard deviations were less than 8.5%. This method is simple, and of good recovery, allowing the detection of nitrobenzene compounds in soil, glass and cotton cloth.

1. Introduction
Nitrobenzene is a kind of common hazardous compounds in emergencies and public safety incidents. All of them are highly toxic and they would pollute the environment after explosion or leakage [1]. Moreover, they could harm human immune system and cause a series of diseases such as anemia, gastritis and hepatitis [2]. Therefore, this kind of substance with high chemical stability is also one of the great concerns for public safety, emergency management, environment assessment. And the establishment of a comprehensive and reliable detection method for the wide range of nitrobenzene compounds is of great significance for the vital interests of the public.

Glass, soil and cotton cloth are the common matrix containing the nitrobenzene compounds on the site of forensic, explosive and environment pollution cases. Although there are some relevant national standards for the detection of nitrobenzene compounds in water and air [3], these standards are not suitable for glass, soil and cotton cloth. Gas chromatography combined with mass spectroscopy and liquid chromatography combined with UV or mass spectroscopy are used to detect the organic explosives residues [4-7]. Liquid chromatography has the advantage of separating the mixed
nitrobenzene compounds due to the stability for nitrobenzene compounds under the low temperature. The technique of liquid chromatography coupled to other detectors have been employed on the analysis of nitrobenzene residues. Perret [6] developed a LC-MS-MS method for the detection of six nitrobenzene compounds. However, this method suffers from high cost, complicated procedures, and poor recoveries from spiked swabs ranging from 78 to 96%. The U.S. Environmental Protection Agency (USEPA) method 8330 could be used as a standard method to identify the nitrobenzene compounds. However, this method is still limited for its poor selectivity, low sensitivity, long time for sample preparation. Thus, a rapid method along with high selectivity and sensitivity is highly desired.

In this paper, we aim to develop a reliable and fast ultrahigh performance liquid chromatographic (UHPLC) method for the determination of 7 nitrobenzene compounds in three different matrixs. This method could increase the accuracy of detection for practical use.

2. Materials and methods

2.1. Materials

All used chemicals were of chromatographical grade (Baker, USA). 2-Nitrotoluene (2-NT) and 3-nitrotoluene (3-NT) were in solid form with purity $\geq 99.0\%$ (Dr. Ehrenstrofer, Germany). 3, 4-Dinitrotoluene (3, 4-DNT) and 2, 6-dinitrotoluene (2, 6-DNT) were in liquid form at 1000 mg/L (O2si, USA); Nitrobenzene (NB), 1, 3, 5-trinitrotoluene (1, 3, 5-TNB), 2, 4, 6-trinitrotoluene (TNT) were in liquid form at 100 mg/L (O2si, USA). Water was purified by the Milli-Q Plus apparatus (Millipore, Bedford, MA, USA).

Stock solutions of 2-NT and 3-NT: around 10.0 mg of these standards was dissolved respectively with acetonitrile and diluted to 10 mL.

The mixed standards solution of the seven nitrobenzene compounds (10 $\mu$g /mL) was prepared through dilution of the seven standards solution with acetonitrile and water (20% acetonitrile) in the same flask.

Calibration standard solutions: mix appropriate amounts of these reference compounds and dilute them with 20% acetonitrile-water solution.

2.2. Instrumentation

The UHPLC system (Waters ACQUITY UPLC, Waters Corporation, Milford, USA) with the UV detector (ACQUITY TUV), was used for chromatographic analysis. The injection volume was 10 $\mu$L. The column (Cortecs Phenyl C18, 2.7 $\mu$m, 2.1×100 mm) was used for separation. Seven nitrobenzene compounds were simultaneously separated and detected through the UHPLC, under the following conditions (Table 1).

2.3. Measurement procedure

Soil, glass, and cotton cloth were dried and homogenized in the tubes. 1.0 g from each collected sample is mixed with 1 mL acetonitrile and ultrasonicated for 20 min. The supernatants were collected after centrifugation. The above extraction process was repeated again and the supernatants were combined. 200 $\mu$L the supernatants was diluted to 1mL with water. The mixtures were filtered with a 0.22 $\mu$m nylon membrane before UHPLC analysis.

3. Results and discussion

3.1. Optimization of extraction solvent

Nitrobenzene compounds can not be dissolved in water, but they could be soluble in benzene, ether, acetonitrile and other organic solvents. Benzene could be used as the extraction solvent or analytical solution according to the China national standard methods for the determination of these compounds in water and air matrix. As the soil, glass and cotton cloth are different from water and air, and benzene is toxic and harmful to environmental. So it is not an ideal extract solvent for nitrobenzene.
Ethyl acetate, methanol, acetonitrile, ethyl ether, dichloromethane and hexane were investigated for good extraction. While dichloromethane is toxic and ethyl acetate, ethyl ether, and hexane are volatile. Methanol and acetonitrile could be ideal extraction solvents. In order to choose the best extraction solvent, standard solution (3 µg/g) was added into 1 g soil, glass and cotton cloth. Each sample was extracted twice by methanol and acetonitrile respectively, then analysed by the UHPLC method. The results showed that recovery efficiency of methanol was 80.3–94.2%, while acetonitrile could achieve 92.6–99.7%. Therefore, acetonitrile was selected as extraction solvent in this experiment.

Table 1. The UHPLC conditions.

| Instruments: | Waters pump; Waters autosampler |
| Columns: | CORTECS phenyl column (2.1×100 mm, 2.7 µm), |
| Column temperature: | 40°C |
| Flow rate: | 0.5 mL/min |
| Total run time: | 10 minutes |
| Mobile Phase: | A: water; B: methanol |
| Gradients: | Time (min) A (%) B (%) μL/min |
| 0.0 | 55.0 | 45.0 | 500 |
| 7.0 | 55.0 | 45.0 | 500 |
| 7.5 | 0.0 | 100.0 | 500 |
| 8.5 | 0.0 | 100.0 | 500 |
| 9.0 | 55.0 | 45.0 | 500 |
| 10.0 | 55.0 | 45.0 | 500 |
| Injection Volume | 10 µL |
| Detector wavelength: | 254 nm |

3.2. Mobile phase
The buffer solution is supposed not to affect the separation of the 7 studied compounds. Therefore, water and methanol are selected as mobile phases. However, the flow rate and the ratio of the mobile phase during the UHPLC analysis is critical for the resolution of the seven nitrobenzene compounds. In order to obtain the best flow rate and mobile phase ratio, flow rates 0.3, 0.4 and 0.5 mL/min, as well as mobile phase ratios 65:35, 60:40 and 55:45 were examined. Results showed that the resolution was getting better with the increased methanol ratio (see Figure 1a, b), especially for 2-NT and 1,3,5-DNT. As the flow rate increased, high resolution of 7 nitrobenzene compounds was obtained (see Figure 1c, d). It turned out that the flow rate 0.5 mL/min, and mobile phase ratio 55:45 made the best separating effect (see Figure 1e). Furthermore, another column (Eclipse PAH, 2.1×100 mm, 3.5 µm) was examined (see Figure 1f) for its performance in separation of these 7 compounds. As it had poor resolution and long running time, we chose the CORTECS phenyl column (2.1×100 mm, 2.7 µm) for chromatographical determination of these 7 compounds.
Figure 1. Chromatograms of the synthetic mixture of standards. (a) Flow rate= 0.5 mL/min, V (water): V (methanol) = 65:35. (b) Flow rate= 0.5 mL/min, V (water): V (methanol) = 60:40. (c) Flow rate= 0.3 mL/min. (d) Flow rate= 0.4 mL/min. (e) Flow rate= 0.5 mL/min, V (water): V (methanol) = 55:45. (f) Column of Eclipse PAH (2.1×100 mm, 3.5 μm). X axis: Time, min.

Figure 2. Chromatograms of the synthetic mixture of standards (0.05 mg/L), Flow rate= 0.5 mL/min, V (water): V (methanol) = 55:45. X axis: Time, min.
3.3. Linearity and sensitivity

Seven kinds of nitrobenzene compounds were separated by UHPLC. The relationship between peak area and concentration of the compounds was investigated to establish a standard calibration curve. The linearity was investigated using the prepared solutions, which were made by diluting 10 mg/L mixed standards solution with 20% acetonitrile-water to 0.05, 0.1, 0.5, 1, 2 and 5 mg/L. The results displayed 0.9999 linear correlation coefficients or better were achieved for most of the standards (Table 2). The chromatogram of 0.05 mg/L standard solution was shown on Figure 2.

The standard solution was diluted until the signal noise ratio was 2~3 times of the base line, the corresponding concentration was set as the instrumental detection limit. 1 g soil, glass and cotton cloth was weighed respectively, and was spiked with standard solution (3~5 times of instrumental detection limit), and further analyzed using the UHPLC method. The concentration of the matrix solution examined was 0.2 mg/L. The limit of detection (LOD) was calculated as 3 times signal noise ratio. LOD and the limit of quantitation (LOQ) for seven standard nitrobenzene compounds were examined at 0.05 mg/L and 0.1 mg/L respectively. In national standards for environmental or surface water, the maximum residue limits for some nitrobenzene compounds in centralized drinking surface water was stipulated. For example, the maximum residue limit for NB is 0.017 mg/L, and TNT is 0.5 mg/L. At present, there is no relevant residue limit in soil, glass and cotton cloth, but based on the above regulations, this method is applicable for detection of nitrobenzene compounds in soil, glass and cotton cloth.

Table 2. Linear equation and correlation coefficient of the seven nitrobenzene compounds.

| Compound | Linear Equations                      | Correlation Coefficient |
|----------|---------------------------------------|-------------------------|
| NB       | y=0.02720361x+0.0005598142           | 0.9999                  |
| 2-NT     | y=0.02255720x+0.0003492071           | 0.9999                  |
| 1,3,5-TNB| y=0.03043708x+0.0005602587           | 0.9999                  |
| 3-NT     | y=0.02950499x+0.0003992283           | 0.9999                  |
| 3,4-DNT  | y=-0.3375308x+0.0003367510           | 0.9999                  |
| 2,6-DNT  | y=-0.5595757x+0.0003767311           | 0.9999                  |
| TNT      | y=-0.1493735x+0.0005167569           | 0.9999                  |

3.4. Analysis in real samples

In order to study the spiked recovery and precision of the method, 1 g soil, glass and cotton cloth was weighed respectively, and further spiked with standard solution (3 µg and 6 µg). Each level was set with 6 replicates. After pre-treatment, the concentrations of these compounds in the matrix solution were 3 mg/kg and 6 mg/kg. Figure 2a and Figure 2b showed the chromatograms of seven nitrobenzene compounds at the spiked concentration of 3 mg/kg and 6 mg/kg in soil. The recoveries of 7 compounds from the three spiked matrices were evaluated after pretreatment and analysis (Table 3). More than 89.2% recovery at 3 mg/kg level and more than 87.8% recovery at 6 mg/kg level were achieved for all the compounds tested. Recoveries between 3 mg/kg and 6 mg/kg were similar in the 7 compounds. 3,4-DNT and 2,6-DNT showed the highest recovery, while NB showed the lowest. The average of recovery was 97.5% in glass, 98.4% in soil and 99.2% in cotton cloth. All the relative standard deviations were less than 8.5% for these compounds. The average of relative standard deviations were 1.98% in soil, 3.09% in glass and 3.56% in cotton cloth. Relative standard deviations between 3 mg/kg and 6 mg/kg were similar in the 7 compounds. These results showed acceptable recoveries and precision requirements for practical application.
Table 3. Recoveries (%) and relative standard deviation (%) in soil, glass and cotton cloth matrices (n=6).

| Compound | Spiked amount [mg/kg] | Soil |  |  |  |  |  |  |
|----------|-----------------------|------|---|---|---|---|---|---|
|          |                       | Recovery | RSD | Recovery | RSD | Recovery | RSD |
| NB       | 3                     | 92.9 | 1.494 | 89.2 | 7.586 | 90.1 | 6.844 |
|          | 6                     | 90.3 | 6.967 | 90.3 | 8.493 | 87.8 | 7.352 |
| 2-NT     | 3                     | 97.9 | 1.747 | 98.4 | 0.977 | 94.8 | 2.928 |
|          | 6                     | 98.6 | 3.358 | 99.4 | 3.709 | 94.9 | 1.594 |
| 1,3,5-TNB| 3                     | 97.1 | 0.991 | 100.3 | 3.169 | 99.3 | 3.503 |
|          | 6                     | 97.9 | 1.875 | 99.7 | 1.967 | 96.9 | 0.763 |
| 3-NT     | 3                     | 92.6 | 3.02 | 94.3 | 1.967 | 90.9 | 2.719 |
|          | 6                     | 97.3 | 1.286 | 94.7 | 4.052 | 92.2 | 1.615 |
| 3,4-DNT  | 3                     | 102.2 | 1.911 | 106.8 | 1.602 | 105.9 | 3.936 |
|          | 6                     | 107.3 | 0.499 | 105.3 | 2.833 | 103.7 | 2.46 |
| 2,6-DNT  | 3                     | 102.1 | 2.944 | 107.7 | 1.548 | 106.9 | 3.601 |
|          | 6                     | 104.7 | 0.574 | 103.6 | 3.701 | 102.4 | 2.443 |
| TNT      | 3                     | 97.1 | 0.991 | 99.9 | 1.349 | 100.1 | 2.217 |
|          | 6                     | 100.3 | 0.096 | 98.9 | 5.065 | 98.5 | 2.2 |

4. Conclusions

In general, the simultaneous and comprehensive analysis of 7 nitrobenzene compounds in soil, glass and cotton cloth samples were established by UHPLC. Nitrobenzene compounds were extracted twice by acetonitrile, and diluted by acetonitrile aqueous solution. Before detection, nitrobenzene compounds were filtered through a nylon membrane. We achieved a good linear correlation coefficients of 0.9999 at the detection range from 0.05 to 5.0 mg/L and LOD of 0.5 mg/kg. Moreover, recovery of all the compounds was more than 87.8%, and the precision was less than 8.5%. This new method could improve the separation efficiencies and resolutions, thus improving sensitivity up to 0.05 µg/mL. This method would provide an efficient technique support for identifying and quantitating nitrobenzene compounds of soil, glass and cotton cloth samples.

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