Method validation for the determination of aniline and 3,3'-dichlorobenzidine by gas chromatography-mass spectrometry

Zhao Wang¹, ², ³, ⁴, *, Risheng Li¹, ², ³, ⁴

¹Institute of Land Engineering and Technology, Shaanxi Provincial Land Engineering Construction Group Co., Ltd
²Shaanxi Provincial Land Engineering Construction Group Co., Ltd
³Key Laboratory of Degraded and Unused Land Consolidation Engineering, the Ministry of Land and Resources
⁴Shaanxi Provincial Land Consolidation Engineering Technology Research Center, Xi'an 710075, China

*Corresponding author: zhao_wang99@shanxidichan.com

Abstract. This article uses and validates the analytical methods for the determination of aniline and 3,3'-dichlorobenzidine in soil by gas chromatography-mass spectrometry, and discusses the determination of semi volatile organic compounds in soil by gas chromatography-mass spectrometry. The feasibility of the application. The results showed that the semi volatile organic compounds aniline and 3,3'-dichlorobenzidine target corresponding to the internal standard response value and concentration showed a good linear relationship, the correlation coefficient was between 0.9997 and 0.9983 respectively; the detection limit was 0.04 mg/kg and 0.04mg/kg, the limits of quantification are 0.2mg/kg and 0.2mg/kg; the relative standard deviations of the two semi-volatile organic compounds in the precision are 2.5~16.9% and 3.6~6.2%, respectively; the accuracy is 2 The recovery rates of these volatile organic compounds were 64.5-83.9% and 72.6-74.3%, respectively. The verification results show that the linear relationship, detection limit, precision and accuracy all meet the requirements of "Determination of Semi-volatile Organic Compounds in Soils and Sediments by Gas Chromatography-Mass Spectrometry" (HJ 834-2017). Using gas chromatography-mass spectrometry technology can achieve fast, accurate and sensitive detection of target substances, and can meet the detection requirements of trace semi-volatile organic compounds, so this method can be better applied to the detection of soil and other environments.

Keywords: soil, SVOC, gas chromatography-mass spectrometry.

1. Introduction

At present, environmental problems are threats and challenges facing the world. With the rapid development of urbanization and industrialization in China, a large number of industrial and agricultural toxic and harmful substances enter the soil, surface water, groundwater and other environments, and the environmental pollution situation is becoming increasingly severe. Semi volatile Organic Compounds
(SVOC) have a direct hazard to the human body and are easy to cause cancer. In recent years, people have gradually paid attention. Semi-volatile organic pollutants are very important environmental pollutants. Some of the more volatile organic compounds are listed as priority pollutants at home and abroad due to their strong "three-inducing" effect, which has a serious impact on the ecological environment and human health. [1-3]. In particular, the occurrence of Changzhou poisonous land and other incidents has accelerated people's consideration of land safety. In order to ensure the safety of land use in the process of site development and utilization, it is necessary to use land engineering, that is, the application of engineering technology to solve land problems, turn unused land into usable land or efficiently use used land, and actively coordinate the harmonious development of human-land relationship Process [4]. It is of great significance to judge and evaluate the risk of pollution [5], efficiently and accurately promote risk assessment and remediation, prevent and control land pollution [6], and provide technical support for controlling the migration and transformation of soil pollutants, improving the ecological environment and land safety. The analysis and detection of soil organic pollution parameters requires the use of high separation efficiency and high sensitivity GC-MS to separate, qualitatively and quantitatively analyze complex samples [7-10]. GC-MS is the earliest instrument to realize the combined technology among the analytical instruments, and is a mature and extremely widely used qualitative and quantitative analysis and testing technology.

2. Experimental design

2.1. Instruments and reagents

GCMS-QP2020NX gas chromatography-mass spectrometer (Shimadzu, Japan); capillary column: 30 m×0.25 mm, 1.4 μm film thickness, 6% cyanopropyl phenyl 94% dimethyl polysiloxane stationary solution; Milli-Q Ultrapure water treatment system (Millipore, USA); One-percent analytical balance (Mettler-Toledo, USA); Rotary evaporator, Soxlet extractor, and nitrogen blowing instrument. Chromatographic grade dichloromethane (Honeywell China Co., Ltd.); Chromatographic grade acetone (Honeywell China Co., Ltd.); Premium pure anhydrous sodium sulfate (Sinopharm Group Chemical Reagent Co., Ltd.); BW900488-1000-A methanol Medium 3, 3-Dichlorobenzidine \(\rho=1000\mu g/mL\) (Altar ink, 1.2mL); 1,4 Dichlorobenzene-D4 in BW901215-2000-A methanol \(\rho=2000\mu g/mL\) (Altar ink, 1.2mL); Nitrobenzene in 90013KM methylene chloride-D5\(\rho=2000\mu g/mL\) (altar ink, 1mL); Aniline in BW90003-1000-A methanol \(\rho=1000\mu g/mL\) (altar ink, 2mL); Helium: high purity grade, purity Above 99.999%.

2.2. Sample processing

Remove the foreign matter in the sample, weigh about 20g (accurate to 0.01g) of the sample in duplicate, one is to measure the dry matter content according to HJ 613, and the other is added with an appropriate amount of anhydrous sodium sulfate, mixed, dehydrated and ground into fine particles. Mix well until loose particles. Transfer all the prepared soil samples into the Soxhlet extraction sleeve, add the intermediate solution of the substitute with the concentration above the middle point of the calibration curve, carefully place it in the Soxhlet extraction reflux tube, and add 100mL dichloromethane to the round bottom solvent bottle. The acetone mixed solvent is extracted for 16 to 18 hours, and the reflux speed is controlled at 4 to 6 times per hour. Then stop heating and reflux, take out the round bottom solvent bottle, and wait for concentration. The rotary evaporator was heated to about 40°C, the extract was concentrated to about 2 mL, and the concentration was stopped. Use a disposable dropper to transfer the concentrated solution to a graduated concentration vessel, and use a small amount of dichloromethane-acetone mixed solvent to rinse the bottom of the rotary evaporator twice, combine all the concentrated solution, and then concentrate with nitrogen blowing, add an appropriate amount of internal standard middle and dilute to 2mL, mix well and transfer to a 2mL sample bottle for determination.
2.3. Instrument reference conditions
Gas chromatographic conditions: inlet temperature: 280°C, splitless; injection volume: 1.0 μl, column flow: 1.0ml/min (constant flow); column temperature: 35°C for 2 min; °C/min increase to 150°C and hold for 5 min; increase to 290°C at 3°C/min and hold for 2min.
Mass spectrometry conditions: electron impact source (EI); ion source temperature: 230°C; ionization energy: 70eV; interface temperature: 280; quadrupole temperature: 150; mass scanning range: 35amu~450amu; solvent delay time: 5min. Data collection method: select ion mode (SIM) mode.

2.4. Calibration curve configuration
Take 5 5ml volumetric flasks, add 2 ml of dichloromethane solvent in advance, measure appropriate amount of semi-volatile organic compound standard intermediate solution, substitute intermediate solution and internal standard intermediate solution, and mix with dichloromethane solvent. Evenly, formulated into a standard series of at least 5 concentration points. The mass concentrations of semi-volatile organics and substitutes were 1.0 μg/ml, 5.0 ug/ml, 10.0 ug/ml, 20.0 μg/ml, 50.0 μg/ml, and the internal standard mass concentrations were all 40.0 μg/ml. According to the reference conditions of the instrument, samples were injected and analyzed sequentially from low concentration to high concentration. The concentration of the target compound is taken as the abscissa; the product of the ratio of the quantitative ion response value of the target compound and the internal standard compound and the mass concentration of the internal standard compound is taken as the ordinate to draw a calibration curve.

2.5. Qualitative and quantitative analysis of samples
Qualitative analysis of the target: qualitatively analyze the target by comparing the retention time, mass spectrum, mass-to-charge ratio and abundance of the target in the sample with the target in the standard series.
Quantitative analysis of the target: Based on the qualitative judgment of the target, the internal standard method is used for quantification according to the peak area of the quantitative ion. When the quantification ion of the target compound in the sample interferes, the auxiliary ion can be used for quantification. Calculate according to the response value of the first characteristic ion of the target and the internal standard. When the first characteristic ion of the target in the sample interferes, the second characteristic ion can be used for quantification.

3. Results and discussion

3.1. Linear range
The measured internal standard response values of aniline and 3,3'-dichlorobenzidine in the soil and sediments were plotted as the ordinate and the concentration as the abscissa to draw a standard curve. The correlation coefficient of each semi-volatile organic compound is shown in Table 1.

| Component name       | Linear equation            | Correlation coefficient | Method requirements | Eligibility   |
|----------------------|----------------------------|-------------------------|---------------------|--------------|
| Aniline              | $Y=4.155635 \times 10^{-2}X-4.40681 \times 10^{-3}$ | 0.9997                  | $R \geq 0.99$       | qualified    |
| 3,3'-Dichlorobenzidine | $Y=7.812631 \times 10^{-2}X-0.1469169$           | 0.9983                  |                     | qualified    |

Linear relationship: The soil and sediment were determined by gas chromatography-mass spectrometry. The internal standard response and concentration of aniline and 3,3'-dichlorobenzidine showed a good linear relationship, and the correlation coefficients were 0.9997 and 0.9983. Meet the requirements of "Determination of Semi volatile Organic Compounds in Soils and Sediments by Gas
Chromatography-Mass Spectrometry" (HJ 834-2017) (the correlation coefficient should be greater than or equal to 0.990).

3.2. Determination of the detection limit and lower limit of the method
According to the relevant regulations of "HJ 168-2010 Environmental Monitoring and Analysis Method Standard Preparation and Revision Technical Guidelines". According to the entire process of sample analysis and testing, the blank experiment was repeated 7 times, the standard deviation of the measurement results of the 7 parallel blank experiments was calculated, and the detection limit of the method was calculated according to formula 1. The data summary table is shown in Table 2.

$$\text{MDL} = \frac{t\sqrt{n-1} \times S}{\sqrt{6}}$$ (formula 1)

MDL—method detection limit; n—number of parallel determinations of the sample; t—t distribution with n-1 degrees of freedom and 99% confidence (one-sided); S—standard deviation of n parallel determinations. Among them, when the degree of freedom is 6, the t value with a confidence of 99% is 3.143.

| Component name       | 1     | 2     | 3     | 4     | 5     | 6     | 7     | Standard deviation | The detection limit | The limit of quantitation |
|----------------------|-------|-------|-------|-------|-------|-------|-------|--------------------|----------------------|------------------------|
| Aniline              | 0.085 | 0.100 | 0.113 | 0.101 | 0.073 | 0.108 | 0.089 | 0.01               | 0.04                 | 0.2                    |
| 3,3′-Dichlorobenzidine | 0.199 | 0.214 | 0.211 | 0.230 | 0.237 | 0.219 | 0.203 | 0.01               | 0.04                 | 0.2                    |

The detection limit: Determination of soil and sediment by gas chromatography-mass spectrometry. The detection limits of aniline and 3,3′-dichlorobenzidine are 0.04 mg/kg and 0.04 mg/kg, and the quantification limit is 0.2 mg/kg and 0.2 mg/kg. Meet the requirements of "Determination of Semivolatile Organic Compounds in Soils and Sediments by Gas Chromatography-Mass Spectrometry" (HJ834-2017).

3.3. Method precision
The laboratory performed 3 sets of horizontal spike experiments on the same sample, and carried out the determination according to all the steps of sample analysis. Each sample was tested in parallel 6 times. The data summary table is shown in Table 3. Calculate the average, standard deviation, relative standard deviation and other parameters of different samples.

| Component name       | 1     | 2     | 3     | 4     | 5     | 6     | AVG   | SD     | RSD%   | Method requirements | Eligibility |
|----------------------|-------|-------|-------|-------|-------|-------|-------|--------|--------|---------------------|-------------|
| Aniline              | 0.085 | 0.100 | 0.113 | 0.101 | 0.073 | 0.108 | 0.089 | 0.02   | 16.9   | 4.7~44              | qualified   |
|                      | 0.428 | 0.469 | 0.440 | 0.465 | 0.427 | 0.407 | 0.439 | 0.02   | 5.5    | 3.9~29              | qualified   |
|                      | 0.800 | 0.853 | 0.831 | 0.848 | 0.850 | 0.855 | 0.839 | 0.02   | 2.5    | 2.9~30              | qualified   |
| 3,3′-Dichlorobenzidine | 0.199 | 0.214 | 0.211 | 0.230 | 0.237 | 0.219 | 0.218 | 0.01   | 6.2    | 4.7~44              | qualified   |
|                      | 0.410 | 0.453 | 0.460 | 0.408 | 0.438 | 0.446 | 0.436 | 0.02   | 5.1    | 3.9~29              | qualified   |
|                      | 0.762 | 0.774 | 0.751 | 0.721 | 0.749 | 0.702 | 0.743 | 0.03   | 3.6    | 2.9~30              | qualified   |

*AVG=Average value; SD=Standard deviation; RSD=Relative standard deviation

Precision: The soil and sediment were determined by gas chromatography-mass spectrometry, and 3 sets of horizontal spike experiments were performed on the same sample for 6 times. The relative
standard deviation of aniline and 3,3’-dichlorobenzidine was 2.5~16.9% and 3.6~6.2%, which meets the requirements of "Determination of Semi-volatile Organic Compounds in Soils and Sediments by Gas Chromatography-Mass Spectrometry" (HJ834-2017) (The relative deviations of the measurement results meet 4.7~44%, 3.9~29%, 2.9~30%, respectively) In the precision requirements.

3.4. Method accuracy
The laboratory verifies the accuracy of the method through a standard addition test. Three sets of horizontal spikes were performed on the same sample, and the determination was performed according to all the steps of sample analysis. Each sample was tested in parallel 6 times. The data summary table is shown in Table 4. Calculate the average value, standard deviation, standard addition amount, standard addition recovery rate and other parameters of the organic substance addition experiment content.

Table 4. Test data of certified reference materials

| Component name | 1     | 2     | 3     | 4     | 5     | 6     | AVG | SD  | BV   | SC   | SRR  | Method requiremen ts | Eligibility  |
|----------------|-------|-------|-------|-------|-------|-------|-----|-----|------|------|------|-----------------------|-------------|
| Aniline        | 0.08  | 0.10  | 0.11  | 0.10  | 0.07  | 0.10  | 0.10| 0.0 | 0.0  | 0.0  | 0.04N | The standard recovery rate is within 47%~119 % | qualified   |
|                | 0.42  | 0.46  | 0.44  | 0.46  | 0.42  | 0.40  | 0.44| 0.0 | 0.0  | 0.0  | ND   |                        |             |
|                | 0.80  | 0.85  | 0.83  | 0.84  | 0.85  | 0.85  | 0.84| 0.0 | 0.0  | 0.0  | 0.04N |                        |             |
| 3,3’-Dichlorobenzidine | 0.19  | 0.21  | 0.21  | 0.23  | 0.23  | 0.21  | 0.22| 0.0 | 0.0  | 0.0  | 0.04N |                        | qualified   |
|                | 0.41  | 0.45  | 0.46  | 0.40  | 0.43  | 0.44  | 0.44| 0.0 | 0.0  | 0.0  | ND   |                        |             |
|                | 0.76  | 0.77  | 0.75  | 0.72  | 0.74  | 0.70  | 0.74| 0.0 | 0.0  | 0.0  | ND   |                        |             |

*BV=Background value; SC=Spiked concentration; SRR=Standard recovery rate

Accuracy: The soil and sediment were determined by gas chromatography-mass spectrometry, and 3 sets of horizontal spike experiments were performed on the same sample. The recovery rates of aniline and 3,3’-dichlorobenzidine were: 64.5~83.9% and 72.6~74.3%, the relative error meets the accuracy requirements in "Determination of Semi-volatile Organic Compounds in Soils and Sediments by Gas Chromatography-Mass Spectrometry" (HJ834-2017) (the spike recovery rate should be between 47% and 119%).

4. Conclusion
The laboratory has verified the two semi-volatile organic compounds detection methods for the determination of soil and sediment aniline and 3,3’-dichlorobenzidine by gas chromatography-mass spectrometry. The volatile organic compounds in the linear relationship correspond to the internal standard response. The value and concentration showed a good linear relationship, the correlation coefficients were 0.9997 and 0.9983 respectively; the detection limits were 0.04 mg/kg and 0.04 mg/kg, and the quantification limits were 0.2 mg/kg and 0.2 mg/kg; the precision was 2. The relative standard deviations of semi-volatile organic compounds were 2.5~16.9% and 3.6~6.2%, respectively; the recovery rates of the two volatile organic compounds in accuracy were 64.5~83.9% and 72.6~74.3%, respectively. The verification results show that the linear relationship, detection limit, precision and accuracy all meet the requirements of "Determination of Semi-volatile Organic Compounds in Soils and Sediments by Gas Chromatography-Mass Spectrometry" (HJ 834-2017). Using gas chromatography-mass spectrometry, the laboratory has successfully verified the detection methods for the determination of soil and sediment aniline and 3,3’-dichlorobenzidine. The methods meet the specified requirements and provide reliable data for environmental monitoring.
spectrometry technology can achieve fast, accurate and sensitive detection of target substances, and can meet the detection requirements of trace semi-volatile organic compounds, so this method can be better applied to the detection of soil and other environments.

References

[1] Liu Zhengtao. Environmental safety and health [M]. Beijing: Chemical Industry Press, 2005: 212 - 223.

[2] RamanathanK, DeblerVL, KosuskoM. Evaluation of control strategies for volatile organic compounds in indoor air [J]. Environmental Progress, 1988, 7 (4): 230 - 235.

[3] OttoD, HundellH, HouseD. Exposure of humans to a volatile organic mixture: I. Behavioural assessment [J]. Archives of Environmental Health, 1992, 47 (1): 23 - 30.

[4] Han Jichang. Introduction to Land Engineering [M]. Beijing: Science Press, 2013: 6 - 7.

[5] Fang Zhilei. The current situation and suggestions of the soil environmental investigation of Shanghai site [J]. Anhui Agricultural Sciences, 2017, 45 (10): 56 - 58.

[6] Wang Ruibo, Chen Yihui, He Liping, et al. Analysis of the main points of environmental supervision work for contaminated site remediation projects [J]. Environmental Science Guide, 2017, 36 (1): 27 - 32.

[7] Qian Min, Liu Jianzhen, Bai Weidong, et al. Application of GC/MS in the food industry [J]. Chinese Condiments, 2009, 34 (9): 101 - 104, 108.

[8] Patrick Roose, Udo A Th Brinkman. Determination of volatile organic compounds in marine biota [J]. J CHROMATOGR A, 1998, 799: 233 - 248.

[9] Huang Yi, Rao Zhu, Wang Chao. Purge and Trap Gas Chromatography-Mass Spectrometry for Qualitative Analysis of Volatile Organic Compounds in Human Urine [J]. Chinese Journal of Analysis Laboratory, 2007, 26(supplement): 64 - 66.

[10] Li Gongke, Hu Yuling, Ruan Huagui. Sample pretreatment instruments and devices [M]. Beijing: Chemical Industry Press, 2007: 207 - 220.