Simultaneous Determination of Aromatic Amines and Pyridines in Soil

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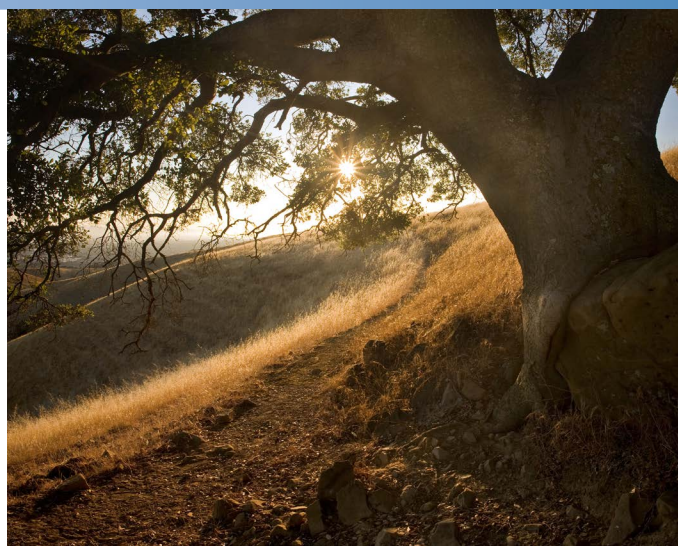
Key Words
Aniline Compounds, Environmental Analysis, Environmental Safety, Acclaim 120 C18 Column, HPLC

Goal
To develop an efficient high-performance liquid chromatography (HPLC) method for fast and simultaneous determination of 2-methylpyridine, 3-methylpyridine, *m*-toluidine, and *p*-toluidine in soil



Introduction
Aniline and pyridines are widely used in the polymer, fuel, pesticide, rubber, and pharmaceutical industries. Not only do some of these compounds have a putrid odor, but they are also suspected potential carcinogens and mutagens that can be highly toxic to life.[1] Therefore, the Ministry of Environmental Protection of the People's Republic of China has proposed a provisional guideline concentration of 0.2 mg/L for pyridine in environmental water.[2] The U.S. Environmental Protection Agency (EPA) lists aniline and 2-methylaniline in its Health Effects Notebook for Hazardous Air Pollutants.[3] As cities grow larger and become more crowded, some of the factories that use these hazardous compounds are closed, and houses and parks are then built on the vacated land. The soil may be polluted, however, and require treatment before building. Therefore, it is necessary to establish a sensitive, efficient, and simple method for the determination of compounds such as pyridines in soil samples.

The most common techniques used for determination of aromatic amine and pyridine compounds in environmental samples are gas chromatography (GC) and HPLC, although capillary zone electrophoresis (CZE) and spectrophotometric methods also have been reported.[1–10] Because the compounds involved are thermolabile and polar, a derivatization step prior to GC analysis is often required—and most such procedures are time consuming and complicated. Therefore, HPLC analysis is a good alternative to GC analysis because derivatization is not needed. Figure 1 shows the structures of four aromatic amine and pyridine compounds that will be simultaneously determined in this study using HPLC.

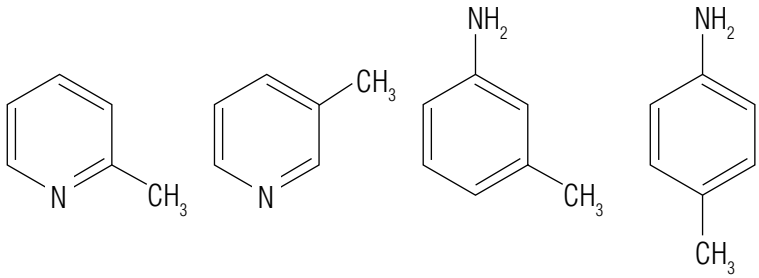


Figure 1. Structures of four aromatic amine and pyridine compounds.

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Equipment, Software, and Consumables

- Thermo Scientific™ Dionex™ UltiMate™ 3000 Rapid Separation LC (RSLC) system, including:
  - LPG-3400RS Quaternary Pump (P/N 5040.0036)
  - SRD-3400 Integrated Solvent and Degasser Rack (P/N 5035.9245)
  - WPS-3000TRS Wellplate Sampler, Thermostatted (P/N 5840.0020), with 25 µL sample loop (P/N 6820.2415)
  - TCC-3000RS Thermostatted Column Compartment (P/N 5730.0000)
  - DAD-3000RS Diode Array Detector (P/N 5082.0020) with 2.5 µL flow cell (P/N 6082.0300)
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2
- Thermo Scientific™ Sorvall™ ST16 Centrifuge (P/N 75004240)
- Thermo Scientific™ Target2™ Nylon Syringe Filters, 0.45 µm, 30 mm (P/N F2500-1)

Reagents and Standards

- Deionized (DI) water, 18.2 MΩ-cm resistivity, generated using a Thermo Scientific™ Barnstead™ GenPure™ Pro ultrapure water system (P/N 50131948)
- Acetonitrile, HPLC Grade (Fisher Scientific P/N AC610010040)
- Methanol, 99.8%, HPLC Grade (Fisher Scientific P/N AC61009-0040)
- Ammonium Acetate, Crystalline/HPLC (Fisher Scientific P/N A639-500)
- Acetic Acid, Optima™ LC/MS (Fisher Scientific P/N A113-50)
- 2-Methylpyridine, 25GR (Fisher Scientific P/N 50-534-017)
- 3-Methylpyridine, >98%, GC (Fisher Scientific P/N 50-014-44238)
- *p*-Toluidine, 99% (Fisher Scientific P/N AC37635-2500)
- *m*-Toluidine, 99% (Fisher Scientific P/N AC15787-0010)

Conditions	
Column:	Thermo Scientific™ Acclaim™ 120 C18, 3 µm Analytical, 2.1 × 100 mm (P/N 059129)
Eluent:	Acetonitrile/ammonium acetate buffer (dissolve 3.85 g of ammonium acetate and 3 g of acetic acid in 1 L of DI water)
Gradient:	0–2 min, 5% acetonitrile; 3.5 min, 80% acetonitrile; 3.6–6 min, 5% acetonitrile
Flow Rate:	0.4 mL/min
Injection Volume:	1 µL (partial-loop injection mode)
Temperature:	35 °C
Detection:	UV, 260 nm

Preparation of Standard Solutions

Stock Standard Mix

Dissolve 0.1 g each of 2-methylpyridine, 3-methylpyridine, *m*-toluidine, and *p*-toluidine in a 100 mL volumetric flask and dilute to volume with methanol. The concentration of each compound will be 1 mg/mL.

Working Mixed Standard Solutions

For calibration, prepare five working mixed standard solutions with different concentrations by diluting the proper amount of each stock standard solution mix with methanol. The volumes of each solution needed to make the calibration standards are shown in Table 1.

Sample Preparation

Soil Sample 1 used in this work was collected from Jinqiao Park in Shanghai, People's Republic of China. Soil Sample 2, also from Shanghai, was supplied by a real estate developer.

Add 10 mL of methanol to 2 g of an air-dried soil sample in a 20 mL centrifuge tube and vortex for 1 min. After ultrasonically extracting the sample at 150 W for 40 min, centrifuge the extract for 2 min at 8000 rpm. Transfer the supernatant to a 10 mL tube and use nitrogen to dry the sample. Add 1 mL methanol to the dried sample. Prior to injection, filter the solution through a 0.45 µm filter.

Table 1. Preparation of working mixed standard solutions.

Vol of 1 mg/mL Stock Standard Mix (mL)	Vol of Methanol (mL)	Final Vol of Working Mixed Standard Solution (mL)	Final Concn of Each Calibration Standard (mg/L)
0.02	9.98	10	2
0.05	9.95		5
0.10	9.90		10
0.20	9.80		20
0.50	9.50		50

To prepare the spiked soil sample, add 5 µL of the 1 mg/mL stock standard mix to 1 g of a soil sample. Prepare the sample as described above. The spike concentrations will be 5 mg/kg for each analyte.

Results and Discussion

Separation of 2-Methylpyridine, 3-Methylpyridine, *p*-Toluidine, and *m*-Toluidine

Initial experiments showed that when using methonal/water or acetontrile/water as the mobile phase, it was difficult to separate *p*- and *m*-toluidine. Therefore, the addition of a phosphate or acetate buffer to the mobile phase was investigated as a way to achieve the separation. The peak resolution between *p*- and *m*-toluidine was 2.4 when using an acetonitrile/acetate buffer mobile phase. In addition to the baseline separation, the peak asymmetry factors of 2-methylpyridine, 3-methylpyridine, *p*-toluidine, and *m*-toluidine were all in the range of 0.92~1.14—better than that yielded when using methanol/acetate, methanol/phosphate, and acetonitrile/phosphate mobile phases. Therefore, an acetonitrile/acetate buffer mobile phase was used in this work. Figure 2 shows a chromatogram of a standard mix of 2-methylpyridine, 3-methylpyridine, *p*-toluidine, and *m*-toluidine under the specified and optimized chromatographic conditions.

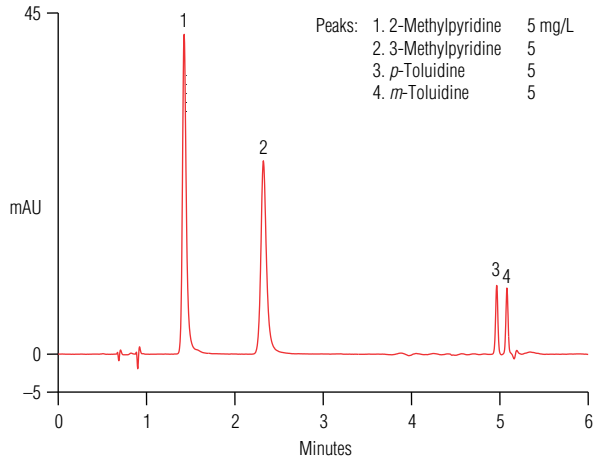

Figure 2. Separation of a mixed standard solution.

Method Reproducibility, Linearity, and Detection Limit

Method precision was estimated by making nine consecutive 1 µL injections of a calibration standard with a concentration of 10 mg/L for each compound. Retention time and peak area reproducibilities (RSDs) are shown in Table 2. Retention time RSDs all are <0.1% and peak area RSDs all are <1.2%, demonstrating good short-term precision for this method.

Calibration linearity of 2-methylpyridine, 3-methylpyridine, *p*-toluidine, and *m*-toluidine was investigated by making three consecutive 1 µL injections of the working mixed standard solution prepared at five different concentrations (i.e., 15 total injections). A linearity range of 2–1000 mg/L for these four compounds was observed when plotting concentration versus peak area. Detailed calibration data calculated by Chromeleon CDS software are shown in Table 2.

Five consecutive 1 µL injections of a working mixed standard solution with a concentration of 2 mg/L for each compound were used for estimating the method detection limit (MDL) using a signal-to-noise ratio = 3. The calculated MDLs also are listed in Table 2.

Table 2. Calibration data and MDLs.

Target Analyte	Retention Time RSD	Peak Area RSD	Regression Equation	r^2	Range (mg/L)	MDL (mg/L)
2-Methylpyridine	0.070	1.117	A = 0.1162c + 1.9669	0.9937	2–1000	0.15
3-Methylpyridine	0.096	1.126	A = 0.1116c + 0.9405	0.9990		0.16
p-Toluidine	0.034	0.707	A = 0.0151c + 0.1036	0.9997		0.50
m-Toluidine	0.035	1.185	A = 0.0137c + 0.0976	0.9996		0.51

Table 3. Sample analysis results.

Soil Sample	Analyte	Detected (mg/kg)	Spiked Sample		
			Added (mg/kg)	Found (mg/kg)	Recovery (%)
2	2-Methylpyridine	Not Detected	5.0	5.1	101
	3-Methylpyridine			4.5	90
	p-Toluidine			3.8	76
	m-Toluidine			4.3	86

Analysis of Soil Samples

2-Methylpyridine, 3-methylpyridine, *p*-toluidine, and *m*-toluidine were not found in the soil samples. Figure 3 shows chromatograms of both soil samples and Soil Sample 2 spiked with four aromatic amines and pyridines. To judge method accuracy, three injections of Soil Sample 2 spiked with 5 mg/kg of each of the standards were made. Analysis results are summarized in Table 3. The average recoveries were in the range of 76–101%, demonstrating that this method can achieve good recovery and is suitable for the determination of aromatic amines and pyridines in soil.

Conclusion

This work uses an ammonium acetate/acetonitrile mobile phase for a rapid, simple, and accurate determination of 2-methylpyridine, 3-methylpyridine, *p*-toluidine, and *m*-toluidine in soil. The separation requires only 6 min and demonstrates good reproducibility and recovery.

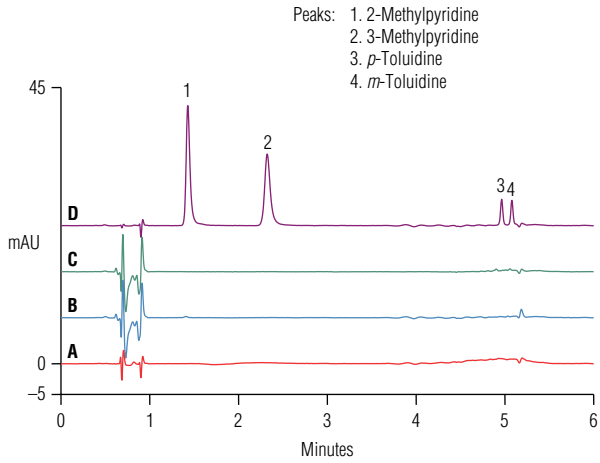

Figure 3. Separation of (A) a blank, (B) Soil Sample 1, (C) Soil Sample 2, and (D) Soil Sample 2 spiked with 5 mg/kg of each standard.

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