



Project Summary

Analytical Procedures for Aniline and Selected Derivatives in Wastewater and Sludge

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A detailed evaluation of gas chromatography (GC) and high performance liquid chromatography (HPLC) for the determination of anilines in aqueous media has been conducted. The most applicable procedure was found to be GC with thermionic nitrogen-phosphorus selective detection (NPD). This GC method is capable of determining a wide variety of anilines at low part per billion (ppb) level in industrial aqueous discharges, as well as effluents from publicly owned treatment works (POTW's). Method performance data for authentic environmental samples are presented. Analytical precision was generally 5-15% Relative Standard Deviation (RSD) and recoveries were generally 75% or better.

This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio, to announce findings of the research project that is fully documented in a separate report of the same title.

Introduction

Aniline and its substituted derivatives (referred to as "anilines") are widely produced for a variety of industrial and commercial purposes, including dyestuff and pesticide manufacturing. The United States Environmental Protection Agency (EPA) has as part of its mission the responsibility for providing test procedures for organic pollutants which are monitored under such regulations as the Clean Water Act (PL-92-500) or Toxic Substances Control Act (TSCA). Since many of the anilines have been placed on the priority list for testing under TSCA, the need arose for an analytical procedure for

determining a wide variety of anilines in industrial wastewater effluents.

This report describes the development and evaluation of GC and HPLC approaches using selective detectors for analyzing trace concentrations in selected industrial wastewaters.

Results and Discussion

The method development effort was conducted as a phased approach wherein each aspect of the procedure was evaluated. The critical elements evaluated included HPLC determinative techniques, GC determinative techniques, extraction approaches, cleanup techniques, and sample preservation. An optimized analytical method, using GC with NPD detection, was devised and evaluated. Details of each evaluation step are described in the main report.

Extraction Methodology

Extraction efficiencies of the anilines were determined in reagent water buffered at pH 5 (0.01 M acetate buffer), pH 7, (0.01 M phosphate buffer), and pH 11 (0.01 M phosphate buffer). Methanol solutions of the compounds were spiked into one-liter volumes of the buffered media in a separatory funnel at the 10 µg/L level. Three successive extractions with 60-mL volumes of methylene chloride were performed using two-minute extraction periods. The extracts were combined and concentrated. Extraction recoveries were determined by HPLC with 254 nm ultraviolet (UV) detection. This extraction approach was selected since existing priority pollutant methods use similar approaches.

GC Column Evaluation

Retention time data for the various anilines on various packed and glass capillary columns were determined. The 10% Carbowax-2% KOH column gave poor performance. Subsequently, a chemically bonded Carbowax column (Ultrabond[®] 20 M distributed by Supelco) was evaluated. All of the anilines were eluted from the Ultrabond[®] column. However, very poor response was obtained for aniline and 4-bromoaniline (indicating loss of these compounds on the column), and a number of compounds coeluted.

The 3% SP-2250 column partially resolved all of the anilines; however, some peak tailing was evident, especially for aniline. The 5% SP-2401 DB (containing KOH) and 1.5% SP-2250/1.95% SP-2401 gave no improvement in peak shape. SP-2250 was chosen as the best available packed column for determination of anilines.

However, each column failed to resolve at least one compound pair. No column completely resolved 3-chloro and 4-chloroaniline although SE-54 appeared to give the best separation for these two compounds. SE-54 was selected for use in the program, although other phases (e.g., SE-52, SP-2100) were also considered to be acceptable. These columns should be considered for use when components coeluting on SE-54 need to be quantified.

During the course of this program, fused silica capillary columns (FSCC) of suitable quality became available. Compared to conventional glass columns, FSCC are durable and easy to use. Retention data for the anilines on two FSCC's are given in Table 1.

Using both SE-54 and SE-30 FSCC, all 19 anilines can be resolved. SE-54 was used as the primary column for the method validation effort although SE-30 probably would have been just as appropriate.

GC Detector Evaluation

The Hall electrolytic conductivity detector (HECD), nitrogen selective mode (NPD), and the photoionization detector (PID) were evaluated in terms of sensitivity using the SE-54 glass capillary column. The HECD and NPD detectors gave similar detection limits in several cases although the NPD was more sensitive for many of the compounds. In addition, the NPD gave a more stable baseline and less day-to-day variation in response. The PID gave very low response for the nitro- and dinitroanilines when the 9.5 eV source was used. Although detection limits were improved considerably when the 10.2 eV

Table 1. Retention Data for Anilines

Compound	Relative Retention Time on Stated Column	
	SE-54	SE-30
Aniline	1.0 (7.5) ^a	1.0 (6.3)
2-Chloroaniline	1.6 (12.1)	1.1 (7.1)
3-Chloroaniline	1.9 (14.6) ^b	1.4 (9.0) ^b
4-Chloroaniline	2.0 (14.7) ^b	1.5 (9.1) ^b
4-Bromoaniline	2.4 (18.0)	1.9 (12.1)
2-Nitroaniline	2.9 (21.9) ^c	2.1 (15.6)
2,4,6-Trichloroaniline	2.9 (21.9) ^c	2.5 (16.3)
3,4-Dichloroaniline	3.0 (22.7)	2.6 (16.6)
3-Nitroaniline	3.3 (24.5)	2.9 (18.0)
2,4,5-Trichloroaniline	3.5 (26.3)	3.2 (20.4)
4-Nitroaniline	3.8 (28.3) ^d	3.4 (21.7)
4-Chloro-2-nitroaniline	3.8 (28.3) ^d	2.9 (22.0)
2-Chloro-4-nitroaniline	4.2 (31.2)	3.3 (24.8)
2,6-Dichloro-4-nitroaniline	4.3 (31.9)	3.5 (26.0)
2-Bromo-6-chloro-4-nitroaniline	4.6 (34.8)	3.8 (28.8)
2-Chloro-4,6-dinitroaniline	4.9 (37.1)	4.0 (30.1)
2,6-Dibromo-4-nitroaniline	5.0 (37.6)	4.2 (31.6) ^c
2,4-Dinitroaniline	5.1 (38.4)	4.2 (31.6) ^c
2-Bromo-4,6-dinitroaniline	5.3 (39.8)	4.5 (33.4)

^a Actual retention time in minutes given in parenthesis.

^b These compounds only partially resolved.

^c These compounds essentially unresolved.

^d These compounds essentially unresolved.

source was used, the higher energy source would result in the detection of virtually all aromatic compounds. Therefore, the PID was not expected to be as specific for anilines as the nitrogen selective detection systems.

The selectiveness of the three detectors were evaluated using an extract from a POTW aqueous effluent. The PID detector (10.2 eV) has a very complex chromatogram with a high baseline level. The chromatographic response using NPD and HECD were similar. NPD was selected for use in the final method because it was more selective than PID and gave better baseline stability and day-to-day repeatability than HECD.

Since the use of both packed and capillary columns was to be considered in the final method, the sensitivity of the NPD for the anilines using 3% SP-2250 packed and SE-54 glass capillary columns was determined.

HPLC Column Evaluation

Although several HPLC columns (e.g., Spherisorb ODS, Lichrosorb C18, Lichrosorb C8, Lichrosorb C2, and μ -Bondapak) was evaluated, Dupont Zorbax ODS was found to give the best resolution for the anilines. Better separation was obtained using 40% acetonitrile/60% acetate buffer than using 50% methanol/40% acetate buffer mobile phase. In addition, the column back pressure was substantially lower when acetonitrile was used. Adjusting the pH of the acetate buffer from 4 to 5.5 in 0.5 pH unit increments

resulted in better separation between 2-chloroaniline and 2-chloro-4-nitroaniline (which coeluted at pH 4 and pH 4.5), but resulted in poor separation between 2-chloroaniline and 4-bromoaniline (which coeluted at pH 5.0 and pH 5.5). Buffer strength had little or no effect on the separation. Based on these results, the optimum HPLC separation system was considered to be a Zorbax ODS column with a 40/60 acetonitrile/0.2 M sodium acetate buffer, pH 4.

HPLC Detector Evaluation

Both UV and electrochemical (amperometric) detectors were evaluated in terms of sensitivity for the anilines. The UV data and electrochemical data for the anilines are presented; all of the nitro-substituted anilines absorb strongly in the 340-400 nm region, whereas the other compounds show no appreciable absorbance above 300 nm. Consequently, the use of UV detection at 350 nm to selectively detect nitroanilines is feasible.

The electrochemical oxidation potentials for the anilines ranged between 0.9 and 1.4 volts, with the dinitroanilines being the most difficult to oxidize. Using HPLC with electrochemical and UV detection, only the nitro-substituted anilines were detected at 340 nm, whereas all of the anilines were detected at 254 and 280 nm. In general, detection limits were 1-10 nanograms injected using UV detection, which corresponded to a level of 0.1-1 μ g/mL in the sample extract.

Extracts from POTW wastewater were injected onto the HPLC-UV system with the UV detector successively at 254, 280, and 340 nm. In all cases, a very complex chromatogram was obtained. Although additional sample cleanup techniques could have been used in an attempt to reduce the background interference level, the evaluation of HPLC analysis was discontinued.

Evaluation of Cleanup Techniques

Two extract cleanup approaches, acid back extraction and adsorption chromatography, were evaluated. Only the most basic anilines (e.g., anilines and the haloanilines) were extracted into 1N H₂SO₄ from methylene chloride. Therefore, acid back extraction was not considered further in this program, although this procedure might be very effective when only aniline or monohaloanilines are to be determined.

Adsorption alumina, basic alumina, and Florisil were evaluated using a variety of elution schemes. Adsorption alumina was found to give very poor recoveries of the anilines even when 10% methanol in ether was used as the elution solvent. Addition of 1% trimethylamine to the 10% methanol in ether solvent did not substantially improve recovery of the anilines. Basic alumina also gave poor recoveries for the anilines when using either the activated or methanol deactivated adsorbent. Aniline recovery was less than 1% using this elution scheme and was only slightly higher (4%) using the methanol deactivation scheme.

The Florisil cleanup schemes gave consistently better recoveries than did the various alumina cleanup schemes. However, the activated Florisil schemes both gave poor recoveries for anilines and the dinitroanilines.

On the basis of these results, the 5% isopropanol deactivation procedure (eliminating the 10% CH₂Cl₂ in hexane fraction) was selected for use in the final method. The isopropanol deactivation procedure was favored because it eliminated the need to prepare adsorbent beforehand and no fines were produced in the deactivation procedure.

Sample Preservation Evaluation

The water samples studied were as follows:

- Reagent water
- POTW water
- Effluent from a coking operation

- Treated (biological treatment) wastewater from a plant producing nitroanilines (wastewater No. 3)
 - Treated (biological treatment) wastewater from a plant producing aniline (wastewater No. 4)
 - Wastewater No. 4 to which 2 mg/L of sodium hypochlorite was added.
- Storage at pH 7 and 4°C were found to give best stability of the anilines.

Method Validation

Based on the results of the experiments described above, an analytical method was selected for validation. This method involved the following steps:

- Extraction of the wastewater at pH 11 with methylene chloride
- Concentration of the extract by Kuderna-Danish concentration
- Cleanup of the extract using Florisil deactivated with isopropanol
- Exchange of the final concentrate into toluene
- Analysis by GC-NPD using an SE-54 FSCC as the primary column. SE-30 FSCC and 3% SP-2250 packed columns are used as secondary columns in the method.

All of the validation work described below involved the use of a SE-54 FSCC. Florisil fractions 1, 2, and 3 (50% methylene chloride in hexane, 5% IPA in hexane, and 5% methanol in hexane, respectively) were combined prior to concentration and analysis.

The analytical curves for the various anilines were determined using reagent water. All steps in the method, including Florisil cleanup, were utilized in this study. Recoveries for the majority of the anilines were constant over the range tested, approximately 3-300 x method detection limit (MDL). However, poor recoveries at less than 10 x MDL were observed for aniline (29%), 4-chloroaniline (7%), and 4-bromoaniline (23%). Nevertheless, all of the anilines were readily detectable at the lowest spike level.

The performance of the method at higher spike levels was evaluated for reagent water, treated wastewater No. 3, and treated wastewater No. 4. Spike levels were approximately 100 µg/L (20-100 x MDL) in all cases. The results for this evaluation are given. Recoveries were generally 70-120% for all samples, except for aniline (55%), and 4-chloroaniline (49%) in distilled water. Percent relative standard deviations were generally 5-15%.

Conclusions

The application of GC-NPD has been found to be an effective method for the

determination of a wide variety of anilines. This approach offers better selectivity and sensitivity than HPLC and other GC detectors. A conventional solvent extraction scheme using methylene chloride gave adequate recovery from aqueous waste samples. A successful adsorption column cleanup technique was identified.

Capillary column chromatography using an SE-54 FSCC offers better sensitivity and selectivity than the optimum packed column (3% SP-2250), although the latter column is still useful as an alternative approach. The optimized analytical method developed gives good precision and accuracy for a variety of water types over a wide range of concentrations. The method reported herein is recommended as the method of choice when a wide variety of anilines are to be determined.

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Stephen Billets was the EPA Project Officer (for present contact see below).

The complete report, entitled "Analytical Procedures for Aniline and Selected Derivatives in Wastewater and Sludge," (Order No. PB 84-140 102; Cost: \$20.50, subject to change) will be available only from:

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