



Project Summary

EPA Method Study 15 Method 605 — Benzidines

Glenn Kinzer, Ralph Riggin, Thomas Bishop, and Cory C. Howard

The U.S. Environmental Protection Agency (USEPA) sponsored an interlaboratory study in which 17 laboratories participated to provide precision and accuracy statements for the proposed EPA Method 605 for the Category 7 chemicals benzidine and 3,3'-dichlorobenzidine (DCB) in municipal and industrial aqueous discharges. Method 605 involves extraction of benzidine and DCB from the aqueous sample at pH 7-8 with chloroform. The extract is then back extracted into acid, reextracted into chloroform at a neutral pH and concentrated. The benzidines are determined in the final extract using high performance liquid chromatography (HPLC) with electrochemical detection. If interference in the measurement of benzidine is encountered, the method provides additional detector settings to increase the selectivity of the analytical system.

The study design was based on Youden's nonreplicate design for collaborative tests of analytical methods. Three Youden pair samples of the test compounds were spiked into six test waters and analyzed. The test waters were distilled water, tap water, a surface water, and three different industrial wastewater effluents. The resulting data were statistically analyzed using USEPA's computer program, Interlaboratory Method Validation Study (IMVS).

This Project Summary was prepared by USEPA's Environmental Monitoring and Support Laboratory, Cincinnati, OH, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back.)

Introduction

USEPA first promulgated guidelines establishing test procedures for the analysis of pollutants in 1973 following the passage of the Federal Water Pollution Control Act in 1972 by Congress. Pursuant to the amendment and publication of these guidelines, USEPA entered into a Settlement Agreement — the Consent Decree—requiring it to study and, if necessary to regulate, 65 "priority" pollutants and classes of pollutants of known or suspected toxicity to the biota. Subsequently, Congress passed the Clean Water Act of 1977 mandating the control of toxic pollutants discharged into ambient waters by industry.

To facilitate the implementation of the Clean Water Act, USEPA selected 113 organic and 16 inorganic toxic pollutants for initial study. The organic pollutants were divided into 12 categories based on their chemical structure. Analytical methods were developed for these 12 categories by USEPA through in-house and contracted research. The use of these analytical methods currently under review by USEPA and industry may eventually be required for the monitoring of the 113 toxic pollutants in industrial wastewater effluents as specified by the Clean Water Act of 1977.

Method 605 was developed in the Battelle-Columbus Laboratories under a contract with the Physical and Chemical Methods Branch, Environmental Monitoring and Support Laboratory-Cincinnati. It is described in the Federal Register, Vol. 44, No. 233, December 3, 1979.

Procedure

The study design was patterned after Youden's nonreplicate plan for collaborative evaluation of analytical methods,

in which samples are analyzed in pairs, each member of a pair having a slightly different concentration of the constituent of interest. The analyst is directed to do a single analysis and to report one value for each sample, as for a normal routine sample. Three Youden pair samples used in this study contained low, medium, and high concentrations of the Category 7 compounds which were spiked into each of six different waters and analyzed.

Prior to the interlaboratory method study, participants analyzed one trial pair of Youden samples to obtain experience with the analytical method and sample handling procedures. Upon completion of the trial analyses, the participants attended a prestudy conference to resolve method interpretation and analytical problems. Finally, participating laboratories were supplied with the test materials required for the formal study and instructed to begin the analyses.

The test waters were:

- a. Distilled water
- b. A municipal drinking water
- c. A surface water, for example, a river, vulnerable to synthetic chemical contamination
- d. Three industrial wastewaters from industries that were potential sources of benzidines.

Analyses were conducted on distilled water to evaluate the proficiency of the analyst to use the method on a sample free of interference. Since municipal drinking and surface waters are subject to contamination, it was considered important to obtain information about the performance of Method 605 in such matrices as well as in industrial wastewater effluents.

Statistical analyses of the data were performed using USEPA's IMVS computer program. The IMVS program developed at Battelle's Columbus Laboratories is a revised version of the USEPA's COLST program and is designed to output the raw data in tabular form and compile summary statistics including:

- Number of data points
- True value
- Mean recovery
- Accuracy as percent relative error
- Overall standard deviation
- Overall percent relative standard deviation
- Single-analyst standard deviation
- Single-analyst percent relative standard deviation.

The overall standard deviations indicate the dispersion expected among values generated from multiple laboratories. The single-analyst standard deviations indicate the dispersion expect-

ed among replicate determinations within a single laboratory.

Results and Discussion

The data collected during this interlaboratory study were statistically analyzed in order to establish the relationship between precision and the mean recovery, and between mean recovery and the true concentration. These relationships are summarized by the linear regression equations presented in Table 1 for (1) overall standard deviation and mean recovery, (2) single-analyst precision and mean recovery, and (3) true concentration and mean recovery.

The slopes of the regression equations can be used to estimate the percentage recovery, and overall and single-analyst percent relative standard deviations at concentrations exceeding 20 µg/L since ignoring the intercepts introduces an error of less than 11 percent in the estimated value. Below 20 µg/L, some regression equations, for example, benzidine in tap water, have intercepts which are rather significant and care should be exercised in using the slopes as an estimate of accuracy and precision.

Based upon the slopes of the equations at concentrations above 20 µg/L, percentage recoveries for benzidine and DCB ranged from 52-70 and 48-66, respec-

tively, for all water types. The overall percent relative standard deviation for benzidine and DCB ranged from 40-69 and 38-58 percent, respectively, for all water types. The single-analyst percent relative standard deviation for benzidine and DCB ranged from 24-40 and 26-40, respectively, for all water types.

Examination of the raw data from each of the laboratories reveals that several of the laboratories did not obtain a recovery greater than 30% for benzidine in any of the matrices, even at the highest level, while many of the other laboratories obtained recoveries consistently above 50% for benzidine. A similar pattern was not found for DCB. This observation indicates that systematic error at several of the laboratories was responsible for low recoveries and high standard deviations of benzidine and DCB.

Oxidants (e.g., chlorine) present in some of the reagent water-sources may have contributed to the low recoveries. Reagent water used for blanks as well as for preparing reagents for sample workup and HPLC mobile phase must be free from even traces of oxidant, as demonstrated by obtaining good recovery of benzidine and DCB from spiked process blanks. Reagent water purified by a multi-cartridge system such as Millipore's "Milli-Q" system, which utilizes reverse

Table 1. Regression Equations for Accuracy and Precision of Method 605 by Compound and Water Type

Water Type	Benzidine	3,3-Dichlorobenzidine
<i>Applicable Conc. Range</i>	(1.00 - 55.00 µg/L)	(1.00 - 70.00 µg/L)
<i>Distilled Water</i>		
Single-Analyst Precision	SR = 0.28X + 0.19	SR = 0.39X - 0.05
Overall Precision	S = 0.40X + 0.18	S = 0.38X + 0.02
Accuracy	X = 0.70C + 0.06	X = 0.66C + 0.23
<i>Tap Water</i>		
Single-Analyst Precision	SR = 0.32X + 0.42	SR = 0.29X + 0.06
Overall Precision	S = 0.50X + 0.21	S = 0.38X + 0.16
Accuracy	X = 0.64C + 0.32	X = 0.64C + 0.12
<i>Surface Water</i>		
Single-Analyst Precision	SR = 0.30X + 0.09	SR = 0.26X + 0.12
Overall Precision	S = 0.56X + 0.06	S = 0.40X + 0.10
Accuracy	X = 0.57C + 0.01	X = 0.59C + 0.14
<i>Wastewater (C-74)</i>		
Single-Analyst Precision	SR = 0.40X + 0.04	SR = 0.27X + 0.28
Overall Precision	S = 0.69X - 0.13	S = 0.56X + 0.13
Accuracy	X = 0.55C + 0.10	X = 0.48C + 0.03
<i>Wastewater (C-75)</i>		
Single-Analyst Precision	SR = 0.34X - 0.09	SR = 0.26X + 0.26
Overall Precision	S = 0.60X - 0.07	S = 0.58X + 0.09
Accuracy	X = 0.62C - 0.18	X = 0.62 + 0.19
<i>Wastewater (C-76)</i>		
Single-Analyst Precision	SR = 0.24X + 0.10	SR = 0.40X + 0.05
Overall Precision	S = 0.64X - 0.01	S = 0.50X + 0.10
Accuracy	X = 0.52C - 0.02	X = 0.53C + 0.08

X = Mean Recovery

C = True Value for the Concentration

osmosis, ion-exchange, and carbon filtration purification steps, is recommended. Carbon filtration is particularly important because it removes residual chlorine. Recoveries for benzidine and DCB from finished drinking water and distilled water were similar, indicating that the dechlorination procedure was effective to prevent oxidation of benzidine spiked into drinking water.

HPLC separation efficiency reported by several of the laboratories was low, in some instances less than 1000 theoretical plates per 25-cm column. Resolution of analytes from interferences becomes a significant problem when such low separation efficiencies are achieved. Probably the method should specify that the minimum separation efficiency greater than 3000 theoretical plates per 25-cm column be achieved before sample analyses are conducted.

Several of the laboratories objected to the large number of manipulations required in the method. This is a valid consideration, although elimination of steps in the procedure does not appear feasible at present.

A statistically significant effect due to water type was established for DCB in the case of wastewater C-74 which yields significantly lower mean recoveries than distilled water. However, this effect was not determined to be of practical importance. There were no other discernible differences due to water types, among mean recoveries, overall precisions or single-analyst precisions.

Conclusions and Recommendations

Based on the results of the interlaboratory method study, Method 605 can be used for measuring concentrations of the Category 7 chemicals, benzidine and DCB, in various water types. Use of Method 605 by experienced analysts should enable industries to meet the permit requirements of the National Pollutant Discharge Elimination System.

Both benzidine and DCB are chemically labile and, hence, the following recommendations should be followed in the workup and analysis of samples.

- Benzidine and DCB are readily oxidized and sources of oxidant must be carefully avoided.
- The basic character of the compounds requires careful pH control throughout the workup procedure in order to obtain maximum extraction efficiencies from aqueous solutions.
- A minimum separation efficiency greater than 3000 theoretical plates per 25-cm column should be achieved

before starting sample analyses.

- The analyst must be well versed in the operational characteristics of the electrochemical detector, which, while relatively straight-forward, is not as widely used as the UV or other common HPLC detectors.

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The complete report, entitled "EPA Method Study 15, Method 605—Benzidines," (Order No. PB 84-211 176; Cost: \$10.00, subject to change) will be available only from:

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