An Expert-Database System for Sample Preparation by Microwave Dissolution. 2. Electronic Transfer and Implementation of Standard Methods

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Currently, implementation of standard methods for chemical analysis relies upon the expertise of an analyst for conversion of the methods into usable procedures and for reproducible execution of these procedures. One can now encapsulate standard methods in a database format which can be transferred among laboratories. These methods can then be converted automatically to reproducible procedures for specific samples types. This paper describes the application of these software techniques to standard methods for microwave sample dissolution.

INTRODUCTION

Performing chemical analyses using standard methods is a major factor in obtaining reliable results. If a standard method is performed exactly as specified for a given sample type, the results of the analysis should be independent of the laboratory and the analyst (within the precision of the procedure and equipment). Validated methods are distributed by professional organizations and government agencies such as the American Society for Testing Materials (ASTM) and the Environmental Protection Agency (EPA). While it has always been hard to maintain quality in the implementation of standard methods, the rapid increase in the number of samples and the decrease in the number of qualified analysts make quality more difficult to achieve.

A significant effort is being expended to develop automated analytical laboratory instrumentation. Two cooperative ventures designed to hasten the development of totally automated laboratories are the Consortium on Automated Analytical Laboratory Systems (CAALS) at the National Institute of Standards and Technology (NIST), Gaithersburg, MD, and the Laboratory Automation Standards Foundation (LASF), Groton, MA. These groups have two critical goals: (1) to develop a generic model for integrating laboratory equipment and instrumentation into automated analytical systems; (2) to specify simple interfaces that allow the components of an automated laboratory system to communicate efficiently. Two analytical projects at NIST, trace elemental analysis and trace organic analysis, are being used to test generic models and communication among laboratory components. To date both projects have focused on sample preparation, the weak link in many analyses.

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SAMPLE PREPARATION

Three important steps in analytical methods are sample preparation, analyte separation, and analyte measurement.[1] While advances in instrumentation have made it easier to standardize the functions associated with separation and measurement, the tasks associated with sample preparation remain largely dependent upon the skill and attention of the analyst. Results from a recent survey[2] indicate that two-thirds of the time required for chromatographic analyses is spent on sample preparation and that approximately half of the errors in these analyses occur in this step. It has been estimated that 90% of inductively coupled plasma analyses require time-consuming, error-prone sample dissolution.[3]

Once the sample preparation equipment and instrumentation have been integrated into an automated laboratory system, methods can be encapsulated and transferred electronically to many laboratories. Software required to capture, transfer, and implement methods for microwave sample dissolution will be described in this paper.

MICROWAVE DISSOLUTION

Until recently, standard dissolution procedures required the addition of acids to samples which were then heated in open vessels on hot plates. Under these conditions, it is difficult to effect standard procedures due to variations in temperature, heating time, evaporation rate, and environmental contamination. In the last 5 years, microwave techniques have proven both effective and efficient. They deliver reproducible amounts of energy for the dissolution of a variety of sample types.[4] These techniques reduce dissolution times from hours to minutes. They also improve the quality of results by providing close control of the dissolution conditions. These characteristics facilitate the standardization of microwave dissolution methods. The acceptance of microwave techniques is shown by the Environmental Protection Agency's recent approval of two standard methods that use microwave energy for analyte release.[5]

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Table I. Vessel Functions for Microwave Dissolution

A *sample* vessel contains the specified amounts of sample and the reagents required for the dissolution procedure.
A *standard* vessel contains specified amounts of reagent(s) and standard materials, such as Standard Reference Materials produced by the National Institute of Standards and Technology, which are used to validate all components in the analytical system. These quality control vessels are treated as samples and identified through the description entered by the analyst.
A *blank* vessel is used to determine the level of analytes in the reagents used for the dissolution. Normally, a *blank* vessel contains the reagents required for the dissolution of the sample but no sample. The number of blanks required for a given procedure, specified as a ratio of samples per blank, is determined by the quality assurance requirements of the procedure and the number of techniques to be used for measurement for analyte concentrations. Blank vessels, interspersed among sample vessels, are treated exactly as any other vessel in the procedure. Blanks for aqueous samples, samples composed of a high percentage of water such as those treated by EPA method 3015, are exceptions: an amount of ASTM reagent-grade water, equal to the amount of sample, is combined with the reagents to form blanks for these aqueous samples.
A *ballast* vessel (power blank) is used to maintain the heating conditions specified in the procedure. They are used, when necessary, to assure that a group of samples contains the number of vessels specified in the procedure. The absorption of microwave energy from a cavity is dependent on the total mass of dielectric absorber in the microwave cavity. The contents of the ballast vessels are identical to blank vessels; however, the contents of ballast vessels are not analyzed.

The direct transfer of microwave energy to the sample provides improved control of the chemistry over more conventional methods of heat transfer. A microwave procedure combines conventional laboratory operations (weighing, reagent addition, etc.) with the conditions for microwave heating (applied power and time). In a closed sample container, the chemical reactions can be replicated without evaporation or exposure to environmental contamination.

The tasks required to implement a microwave procedure are (1) sample loading, (2) reagent addition, and (3) microwave heating. The first task always requires an analyst, while the latter two tasks can be automated. Vessels may be classified according to their function [sample, calibration, blank, or ballast (Table I)]. Vessels that undergo the same dissolution procedure belong to a set known as a *batch*. Vessels heated simultaneously in the microwave unit belong to a set known as a *group*. A single batch of vessels may contain several groups. Samples in our analytical setup are usually processed in batches.

Dissolution procedures may require one or more *steps*. A step is defined as the addition of acid(s) to a vessel followed by heating the vessel in the microwave unit. The first step of a multistep process consists of removing the cap, adding reagent(s), capping, heating, and cooling. If the procedure contains a second step, the vessel is then uncapped, additional reagents are added, and the vessel is capped, heated, and cooled.

The microwave heating is programmed in energy *increments* characterized by two variables: (1) the absolute power (watts) to be applied during the increment and (2) the length of the increment (seconds). In order to sustain the temperature required for a dissolution procedure, an energy balance must be maintained between the microwave energy transferred to the vessels and the heat lost from the vessels. The power required to reach and maintain the temperatures specified for a dissolution procedure is a function of the following parameters: (1) the identity, concentration, and amount of solvent (acid); (2) the physical and material characteristics of a vessel; and (3) the number of vessels in the group. During the heating process several different power settings may be employed to maintain the proper energy balance.

In order to perform a standard procedure, a microwave unit must be calibrated.[6] Calibration permits the watts stipulated in the procedure to be converted to power settings (%) for the specified microwave unit. A detailed discussion of microwave calibration procedures is given in refs 3 and 5.

Figure 1 shows the temperature profile for the microwave decomposition of a batch of 20 vessels (four groups of five vessels) by EPA method SW 846 3015 (The Resource Conservation and Recovery Act, RCRA). Note the single-step procedure contains three different power settings (increments) and that the reactions are reproducible over four different runs. The EPA is also in the process of approving microwave, acid-assisted digestions for sediments, sludges, soils, and oils (method 3051).[7,8] These methods release metal analytes from the matrix by hot-acid leaching. By contrast, total decomposition methods destroy the entire matrix. As a result, the results of elemental analysis from samples processed by these leach procedures will be different from corresponding results from the elemental analysis of samples undergoing total decomposition. Therefore, the ability to control and reproduce experimental conditions is a critical factor in standard methods for leaching and decomposition of samples. It also follows that the electronic transfer of conditions is an important factor in standardizing these methods among laboratories.

GENERATION OF A MICROWAVE PROCEDURE FILE

A microwave procedure must contain values for the following dissolution parameters required to reach and maintain the temperature(s) specified for the dissolution:

- sample mass
- identity and volume of the acid(s)
- size and type of vessel
- number of vessels in a group
- heating program (watts and times)

The principal factors influencing values of these parameters are

- total mass of material in the microwave cavity
- sample matrix
- identity and expected concentration level of the analyte(s)
- technique to be used for analyte measurement

Once these factors have been identified, an experienced analyst can determine a set of values for the dissolution parameters. For example, sample amounts for organic sample matrices (which produce larger volumes of gases upon dissolution) should be smaller than amounts of inorganic samples (which produce little or no gas upon dissolution). A less experienced analyst can predict these values using an expert system.[9] In either case, the choice of parameters must be validated by successfully performing the dissolution with the predicted parameters. Details for the validation of EPA methods are given in refs 5–7.

When a given procedure has been validated, values for the dissolution parameters are captured in a database. The analyst can then use the dBASE III (or Clipper) program described below to generate the conditions for the current batch of samples and laboratory equipment from a method stored in the database (Figure 2). These conditions are written to the microwave procedure file in the form of a set of values for the dissolution parameters. This file can then be used to execute the dissolution procedure in any of three modes [manual, semi-automated, and fully automated (Figure 3)]. In summary, the database provide a means of encapsulating and transferring

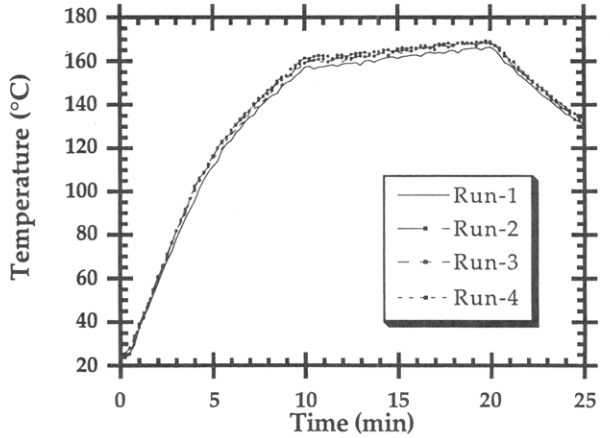

Figure 1. Reproducibility of reaction conditions within microwave vessels using EPA method 3051.

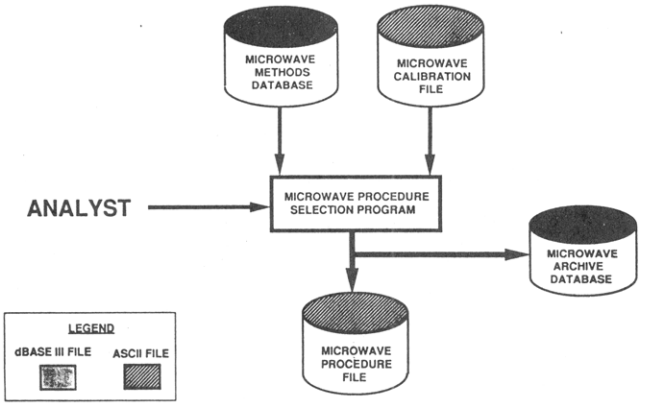

Figure 2. Selection of microwave procedure and creation of procedure file.

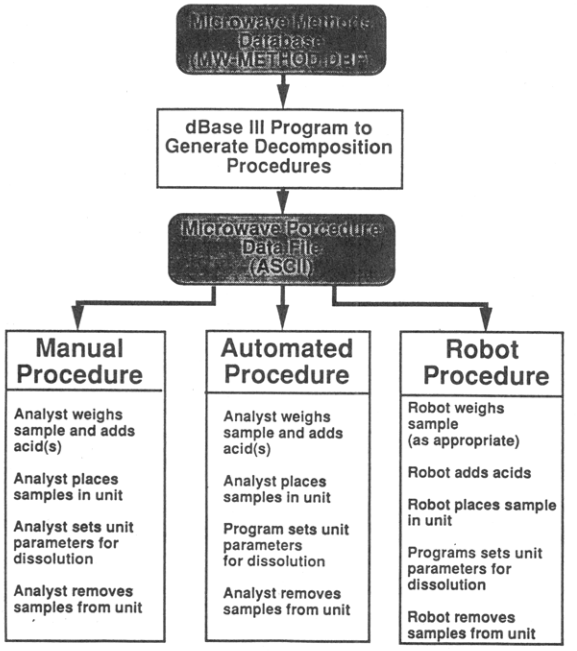

Figure 3. Electronic transfer of standardized procedures for microwave acid dissolution.

microwave methods in specific protocols for a variety of sample types and laboratory equipment.

MODES FOR MICROWAVE DISSOLUTION

In the manual mode, detailed directions for all tasks associated with sample preparation and microwave heating are displayed for the analyst. Printed instructions customized

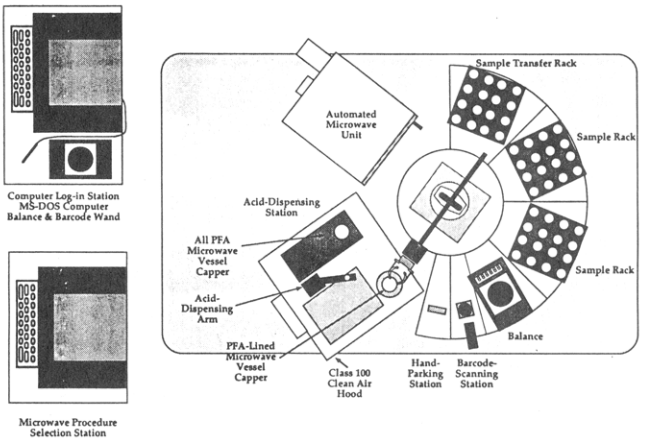

Figure 4. Configuration of microwave sample preparation station.

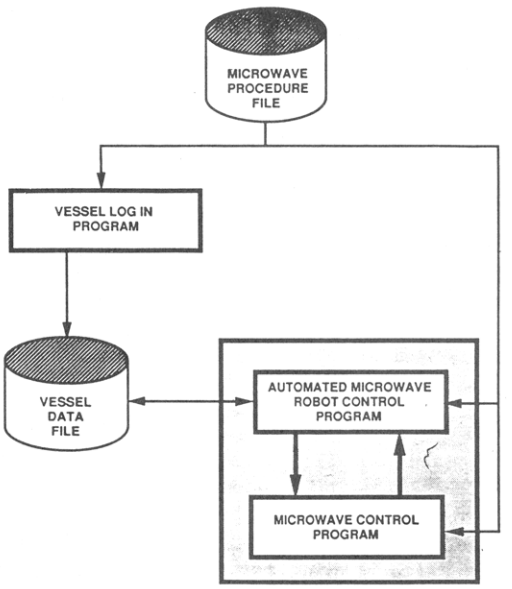

Figure 5. Software for the automated microwave sample preparation station.

for the current batch of samples are also available. In the semiautomated mode, the analyst receives directions for preparing samples and placing them in the microwave unit. The program that operates the automated microwave unit retrieves the instructions for heating from the procedure file and transfers the instructions to the unit. The heating conditions for the dissolution method are combined with values for the specified microwave unit to produce the required temperatures for the method.

In the fully automated mode, information from the procedure file is accessed by programs responsible for directing the robotic system and the microwave unit.[10] The totally automated dissolution system consists of three major components, a sample entry station, a robot with sample preparation equipment, and a programmable laboratory microwave unit.[11] The analyst operates the sample entry station, while the latter two components are fully automated. The analyst enters sample, standard, blank, and ballast vessels into the system and positions them in a rack accessible to the robot (Figure 4). The robot moves samples to the positions required for addition of reagents and transfer of vessels to and from the microwave unit. Reagents are added in a small clean air hood to maintain the integrity of the analysis. The microwave unit operates with the robot system to perform the transfer and heating of vessels. Current information on each vessel is stored in the vessel data file (Figure 5). Thus, the system operates under controlled, reproducible conditions to produce

Table II. Batch Specific Information[a]

BATID	identification of the current batch of samples
LASTN	last name of the analyst who is entering the current batch of samples into the system
FIRINT	analyst's first initial
MIDINT	analyst's middle initial
DAT	date the samples were logged into the system
UNITID	identification of microwave unit
NO_SAMP	number of samples
NO_BLANKS	number of blank vessels
NO_CALIB	number of calibration vessels
NO_BALLAST	number of ballast (power blanks) vessels
NO_VESS	total number of vessels (sample, calibration, blank, and ballast)

[a] Values for the first seven fields are entered directly by the analyst when samples from the batch are entered, and the values of the last three fields in this section are calculated by the microwave procedure program. Note: dBASE III has a 10-character limit on field names.

batches of dissolved samples, standards, and blanks.

The sample entry station required for fully automated operation is optional for the other two modes. The sample entry program reads the procedure file and generates screens that direct the analyst to place the specified amount of sample into the vessel. After the vessel has been loaded, the program confirms that the sample weight is correct for the current procedure. If the sample weight is correct, the analyst uses a barcode reader to record the identity of the vessel and places the vessel in the rack position specified by the program. Data from the balance and the barcode reader are then transferred directly to a vessel data file created by the program.

Generating a standardized procedure file for a specific batch of samples from a database containing validated dissolution methods is an efficient means to transfer standard methods. This electronic transfer of standard methods can improve the quality of information obtained in a variety of laboratory environments ranging from manual to totally automated analyses. Electronic transfer is an attractive alternative to printed methods for microwave sample dissolution. It can be used for many other analytical methods in the future.

MICROWAVE DISSOLUTION DATABASE

The MW-METH.DBF database file includes validated procedures for microwave acid-dissolution methods. Although each record contains a unique procedure, several instances may exist for a given method because the power requirements depend upon the number of vessels in a group. Thus, two instances could exist for the dissolution of oil samples, one each for groups of four and six vessels. Although these procedures (instances) apply different amounts of microwave energy to the groups of samples, they produce identical dissolution conditions.

A record in the MW-METH.DBF file is divided into four types of fields according to the information they contain. The initial set of fields (Table II) is used for temporary storage of information entered by the analyst to describe the current batch of samples. The second set of fields (Table III) contains general information for a given procedure. The third set of fields (Table IV) contains the detailed information required to perform a single step of the procedure. A final set of fields (Table V) contains archival information on the origin of the procedure.

A multistep procedure must contain one record for each step of the procedure. The step-specific sections of records for a multistep procedure will vary with each step. All other information in these records remain constant. Although this format requires that many fields have to be repeated, it allows the structure of the MW-METH.DBF file to be consistent with the structure of the microwave procedure file. The dBASE III editor is used to enter data on validated procedures into the MW-METH.DBF file.

Table III. General Procedure Information

PROC_ID	numerical identification of the method, e.g., 3015.01
PROC_NAME	name of the method, e.g., EPA aqueous samples and extracts
PROC_STEPS	number of steps in a given procedure
SAMP_AMT	quantity of an individual sample
SAMP_UNITS	units of measure for the quantity of sample specified by SAMP_AMT
SMP_TOL	tolerance for the sample quantity given in the units specified by SAMP_UNITS
VESSEL	vessel type and volume to be used in the current procedure, e.g., 120-mL Teflon PFA or 120-mL Teflon-lined vessel
VESS_MASS	average mass in grams for the vessel and cap assembly to be used in the current procedure
VESS_TOL	tolerance in grams for the mass of the vessel and cap to be used in the current sample protocol
CAP_VESSEL	description of the type of cap to be placed on the vessels (some caps have additional fittings)
S_PER_B	ratio of samples to blanks required by the method
S_PER_C	number of samples per calibration required by the method
V_PER_GR	number of sample, blank, or ballast vessels to be heated in the microwave unit as a group
MAXPOWER	maximum power (watts) required for the current procedure
F_SMP_TOL	maximum weight difference permitted between the weights of the sample before and after heating in the microwave unit

Table IV. Step-Specific Information

NO_INCREM	number of increments in the microwave heating program for the current sep
POWER1[a]	power (watts) required for the first increment of the heating program for the current step
PERCENT1[a]	% power setting for the first heating increment for the current microwave unit; values of the power settings must be determined prior to each analysis for each individual microwave unit by the specified calibration procedure
TIME1[a]	length of time (s) of the first heating increment
TEMP1[a]	temperature (°C) to be reached at the end of the first heating increment
PRESS1[a]	pressure (atm) to be reached at the end of the first heating increment

[a] These fields are repeated in each of the four possible heating increments regardless of the actual number of increments in the procedure.

Table V. Archival Information

ANALYST	analyst responsible for the development of the current procedure
DATE	date the current procedure was validated
LAB	identity of the laboratory where the current procedure was developed and validated
C_UNITID	identification of the unit used to develop and validate the procedure
UNITID	identification of the unit used for the current batch of samples
LASTN	last name of the analyst responsible for the current procedure
FIRINT	first initial of the analyst
MIDINT	middle initial of the analyst

MICROWAVE PROCEDURE FILE

This ACSII text file contains all of the information required to process a batch of samples with a standard dissolution procedure using a specified, calibrated microwave unit (Figure 5). It is created by the microwave procedure selection program (a dBASE III program) using information from the analyst, the MW-METH.DBF file, and the microwave calibration file (Figure 2).

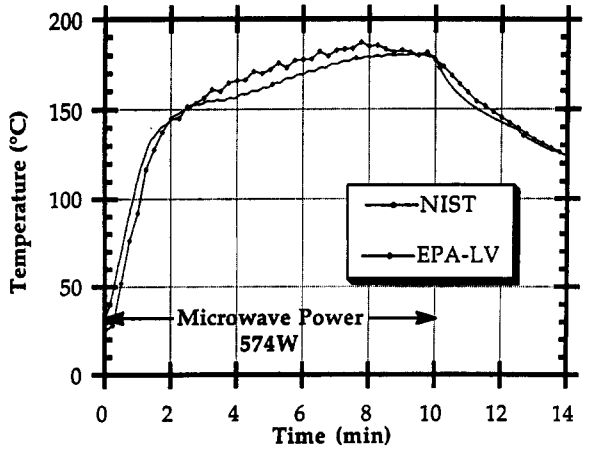

Figure 6. Comparison of EPA method 3051 temperature profiles using identical control software.

To generate a procedure file, the analyst enters the following information into the microwave procedure selection program (Figure 2): the identification of the microwave unit, his or her name, the batch identification, and the number of samples in the batch. The analyst then selects the dissolution method from a menu of methods available in the MW-METH.DBF file. The program compares the power requirements for each procedure with the maximum power available from the specified unit and displays the number of vessels in a group for those procedures that can be run by the unit. For example, a procedure for groups of 10 vessels require more power than procedures for groups of six or eight vessels. Thus, the unit selected by the analyst might have sufficient power to run the six- or eight-vessel procedures but not the 10-vessel procedure. In this case the 10-vessel procedure would not appear on the menu and the analyst's options are limited to procedures for either six or eight vessels.

The microwave procedure selection program (Figure 2) then calculates and displays the batch-specific information shown in Table II. The power settings for the microwave unit are calculated using data for the specified unit from the calibration data file and the absolute power for the heating increments from the MW-METH.DBF file. These setting are displayed to the analyst.

This program also creates the microwave procedure file [in ASCII format (Figure 2)] that contains the information required to process the batch of samples using a standard dissolution method. (This procedure is an instance of the method.) The structure for the microwave procedure file is identical to that of a record in the microwave procedure database with one exception, the fields of the archive section are omitted. The fields of the microwave procedure, delimited by commas, appear in the same order as those in the records of the MW-METH.DBF file. Information on the composition and conditions used to process each batch is permanently recorded in the microwave archive database file (Figure 2).

Laboratories at NIST and EPA Las Vegas have used this system to reproduce the conditions for EPA method 3051 on calibrated microwave units. The following procedure has been successfully duplicated in both laboratories. Six samples were prepared manually following the directions contained in the database and placed into the microwave unit. The samples were digested by using the unit-specific microwave conditions. The temperature in one digestion vessel was monitored in each laboratory (Figure 6). The ability to transfer standard methods between laboratories is shown by the identical temperature profiles.

CONCLUSIONS

Validated methods for microwave sample dissolution can be stored in dBASE III files and transferred electronically among different laboratories. Analysts can use a dBASE III (or Clipper) program to convert these general methods into detailed procedures for the dissolution of specific batches of samples in calibrated microwave units. A procedure stored in an ASCII text file can be read by different programs to direct microwave sample dissolution. The dissolutions may be carried out in either a manual, a semiautomatic, or a totally automated mode depending upon the identity and configuration of the laboratory equipment. The same microwave procedure file can be used by different programs for sample dissolution in any of the above operation modes.

The transfer of these methods between the NIST and EPA Las Vegas laboratories proves the feasibility of electronic transfer of microwave dissolution methods among laboratories with calibrated microwave units. Three microwave units capable of being programmed from an external computer are now testing the transfer of methods in the semiautomated and totally automated modes of operation. These systems are located at the National Institute of Standards in Gaithersburg, MD, the EPA Environmental Methods Support Laboratory in Las Vegas, NV, and the EPA Region 10 Laboratory in Seattle, WA. The EPA microwave methods are being transferred among these laboratories using the database structure and programs described in the preceding sections.

The ability to transfer validated methods in this manner will be evaluated and the results reported in a future paper. At present only the three systems cited above are capable of using microwave procedures generated from the database files. If this method of electronic transfer proves feasible for microwave methods, these principles can be used to develop databases and programs required to transfer a variety of other analytical methods.

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