AOCS Procedure M 1-92
Replaces M 1-59 • Reapproved 2017

Determination of Precision of Analytical Methods

The former AOCS Procedure M 1-59, "Determination of Precision of Analytical Test Methods," was declared a surplus (i.e., outdated) method in 1989. Current guidelines for the statistical analysis of collaborative study data have resulted from harmonization efforts between the AOAC International and International Union of Pure and Applied Chemistry (IUPAC) and the International Organization for Standardization (ISO).

AOCS Procedure M 4-86, revised and amended in 1988 and 1992, explains collaborative study procedures and reviews statistical parameters in detail.

The accepted statistical method for the analysis of collaborative study data is ISO 5725:1994. The minimum statistics to be determined and reported for any collaborative study, and which should be included in the AOCS method, are repeatability (r) statement, reproducibility (R) statement, and table of statistical parameters (as noted in Table 1). Specifically, the statements and table that should appear in the collaborative study report and the AOCS method based on the collaborative study are as follows:

Repeatability limit—The absolute difference between two independent single test results, obtained with the same method on identical test material in the same laboratory by the same operator using the same equipment within short intervals of time, should not be greater than the repeatability limit (r) as calculated from the formulas in Table 1.

Reproducibility limit—The absolute difference between two single test results, obtained with the same method on identical test material in different laboratories with different operators using different equipment, should not be greater than the reproducibility limit (R) as calculated from the formulas in Table 1.

The reader may consult the references cited at the end of AOCS Procedure M 4-86. The ISO 5725:1994 protocol, summarized in References 2 and 3, includes sequential application of the Cochran and Grubbs tests at 2.5% probability until no further outliers are flagged or until a drop occurs of 22.2% (2/9) of the original number of laboratories providing data. The AOACI Inter-laboratory Statistical Program is available from AOAC International (www.aoac.org).

REFERENCES

1. Pocklington, W.D., *Pure Appl. Chem. 62:*149 (1990).
2. Horwitz, W., *Pure Appl. Chem. 67:*331 (1995).
3. Collaborative Study Guidelines, (www.aoac.org)

TABLE 1

The interlaboratory study completed by the AOCS [Name of Technical Committee] in [year], in which [number] laboratories participated, each obtaining [number of determinations] test results for each sample analyzed at [note any special conditions, e.g., temperature], gave the statistical results (evaluated in accordance with ISO 5725-1994) summarized in the following table.

	Sample(s)[a]	
	A	B
Number of laboratories retained after eliminating outliers		
Number of outlying laboratories removed		
Mean value(s), x [Note units associated with mean value, e.g., %, µg/kg, etc.]		
True or accepted value, if known		
Repeatability standard deviation (S_r)		
Repeatability relative standard deviation (RSD_r)		
Repeatability value, $r = (2.8 \times S_r)$		
Reproducibility standard deviation (S_R)		
Reproducibility relative standard deviation (RSD_R)		
Reproducibility value, $R = (2.8 \times S_R)$		

[a]Sample key: A, [description of sample]; B, [description of sample]; (list all samples analyzed, both in Table and in sample key).

AOCS Laboratory Safety

INTRODUCTION

The following sections do not contain complete listings of all the elements involved in laboratory safety. These precautionary notes serve as a reminder of possible hazards involved in the use of particular operations or substances, especially those items and materials frequently used in AOCS methods. The user of these methods should refer to standard texts on laboratory safety for a more complete treatment of the subject. Follow safety requirements and rules issued by voluntary organizations and government agencies [Occupational Safety and Health Administration (OSHA), in particular] expert in the field of laboratory safety.

EQUIPMENT

Blenders, grinders, electrical equipment—Motors on high-speed blenders used to mix flammable solvents with other material should be rated for use with the materials in question and in the class and division rating of the lab where the work is being performed. Blend toxic or flammable liquids in an effective fume-removal area. Accidents involving electrical equipment may result in mechanical injury, e.g., fingers are being caught in chopping mill knives or grinders; electrical shock, which may be due to lack of or improper grounding, defective equipment, exposed wiring, or inadequate maintenance; and fire through ignition of flammable vapors by electrical sparks. Ground all electrical equipment. Installation, maintenance, and repair operations should be performed by qualified electricians.

Atomic absorption spectrophotometer—Use effective fume-removal device to remove gaseous effluents from burner. Use specially designed exhausts when nitrous oxide (N_2O) is used as a fuel oxidant. If instrument has drain trap, check to ensure it is filled with water before igniting burner. Explosions of fuel gas accumulated through drain traps have been reported.

Compressed gas cylinders—Identify contents (by means of attached decal, stencil or tag) of compressed gas cylinders by name of gas contained in the cylinder rather than by color codes. Secure cylinders in upright position by means of strap, chain, or nontip base. Use only correct pressure gages, pressure regulator, and flow regulator for each size of gas cylinder and type of gas, as specified by supplier. Use toxic gases only in effective fume-removal areas. When burning gas or performing a reaction, use back flow prevention device in gas line to prevent flame or reaction from being sucked back into cylinder.

Distillations, extractions, evaporations—For flammable liquids, perform operations behind safety barrier with hot water, steam, or electric mantel heating. Do not use open flames to heat flammable liquids. Use effective fume-removal device to remove flammable vapors as they are produced. Set up apparatus on firm supports and secure all connections. Leave ample headroom in flask and add boiling chips before heating begins. All controls, unless vapor sealed, should be located outside vapor area. For toxic liquids, use effective fume-removal device to remove toxic vapors as they are produced. Avoid contact with skin and inhalation of vapors. Store and dispose of toxic solvents in the manner prescribed by the Environmental Protection Agency (EPA) and OSHA.

Vacuum—Any apparatus to be used under vacuum shall be coated, taped, or otherwise treated to minimize effects of possible implosion, and a safety shield in place during operation. Vacuum pump drive belts must have effective guards.

ACIDS

Use effective acid-resistant fume-removal device whenever heating acids or performing reactions which liberate acid fumes. When diluting acids, always add acid to water, unless otherwise directed in a method. Keep acids off skin, and protect eyes when working with acids. If acids come in contact with skin or eyes, wash immediately with large amounts of water. Do not store oxidizing acids (perchloric, nitric, sulfuric) near organic materials. Mixing organic materials with these acids, particularly perchloric, could result in an explosion.

Acetic acid in the pure state is moderately toxic by ingestion and inhalation. It is a strong irritant to skin and tissue. The TLV in air is 10 ppm.

Hydrochloric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is toxic by ingestion and inhalation and a strong irritant to eyes and skin. The use of a properly operating fume hood is recommended. When diluting the acid, always add the acid to the water, never the reverse.

Hydrogen bromide gas and hydrogen bromide are toxic by inhalation and strong irritants to eyes and skin. Use a properly operating fume hood when working with these compounds.

Nitric acid is a highly corrosive and toxic oxidizing agent. Use effective acid-resistant fume-removal device whenever heating acids or performing reactions that liberate acid fumes. When diluting acids, always add acid to water unless otherwise directed in a method. Keep acids off skin and protect eyes when working with acids. If acids come in contact with skin or eyes, wash immediately with large amounts of water. Do not store oxidizing acids (perchloric, nitric, sulfuric) near organic materials. Mixing organic materials with these acids, particularly perchloric, could result in an explosion.

Periodic acid is an oxidizing agent and is dangerous in contact with organic materials. It is a strong irritant. It decomposes at 130°C. Do not use cork or rubber stoppers on storage bottles.

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. It is a dehydrating agent and should not be stored in the vicinity of organic materials. Use great caution in mixing with water due to heat evolution that can cause explosive spattering. Always add the acid to water, never the reverse.

ALKALIES

Alkalies can burn skin, eyes, and respiratory tract severely. Wear heavy rubber gloves and face shield to protect against concentrated alkali liquids. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials, like sodium hydroxide and potassium hydroxide, always add pellets to water and not vice versa. These alkalies are extremely exothermic when mixed with water. Take precautions to contain the caustic solution in the event the mixing container breaks from the extreme heat generated.

Potassium hydroxide can severely burn skin, eyes, and respiratory tract. Wear heavy rubber gloves and face shield to protect against concentrated alkali liquid splash. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials, such as potassium hydroxide, always add the pellets to the water and not the reverse.

Sodium hydroxide can severely burn skin, eyes and respiratory tract. Protective clothing should be worn when working with this alkali. Wear heavy rubber gloves and face shield to protect against concentrated alkali liquid splash. Use effective fume-removal device or gas mask to protect respiratory tract against alkali dusts or vapors. When working with extremely caustic materials, such as sodium hydroxide, always add pellets to water and not the reverse.

SOLVENTS

Vapors from some volatile solvents are highly toxic. Several of these solvents are readily absorbed through the skin. Do not let vapors concentrate to a flammable level in the work area, because it is nearly impossible to eliminate all chances of sparks from static electricity, even though electrical equipment is grounded. Use effective fume-removal device to remove solvent vapors as they are liberated.

Acetone is a highly flammable solvent. Forms explosive peroxides with oxidizing agents. Use effective fume-removal device. Do not mix with chloroform.

Acetonitrile is a flammable solvent. There is toxic action by skin absorption and inhalation. A fume hood should be used at all times when using acetonitrile.

Aniline is an organic chemical compound consisting of a benzene ring attached to an amino group. Acute exposure can cause upper respiratory tract irritation and congestion.

Benzene is a highly toxic and highly flammable solvent. Avoid contact with the skin. Do not breathe vapors. Use effective fume-removal device. Decomposes violently in the presence of strong oxidizing agents. Reacts violently with chlorine. Benzene is a cancer-causing agent.

Carbon disulfide is a colorless, highly-flammable poisonous liquid. It is harmful either by inhalation, prolonged or repeated skin contact, or by ingestion. Chronic poisoning may ensue from repeated exposure to vapor. It is a dangerous fire and explosion risk, and can be ignited by friction. Extreme precautions should be taken when using this solvent. A fume hood should be used at all times when handling this solvent.

Carbon tetrachloride is a known carcinogen. It is toxic by ingestion, inhalation, and skin absorption. It is a narcotic. It should not be used to extinguish fires. It decomposes to phosgene gas at high temperature. It reacts violently with alkali metals. A fume hood should be used at all times when handling this solvent.

Chlorobenzene is a colorless flammable liquid. It has a moderate fire risk. Explosive limits are 1.8–9.6% in air. Avoid inhalation and skin contact. The TLV is 75 ppm in

air. Chlorobenzene and trichlorobenzene are toxic by ingestion and inhalation. Use a properly operating fume hood when working with this solvent.

Chloroform is a known carcinogen. It is toxic by inhalation and has anesthetic properties. Avoid contact with the skin. Prolonged inhalation or ingestion can lead to liver and kidney damage and may be fatal. It is nonflammable, but will burn on prolonged exposure to flame or high temperature, forming phosgene gas when heated to decomposition temperatures. Can react explosively with aluminum, lithium, magnesium, sodium, potassium, disilane, N_2O_4, and sodium hydroxide + methanol. The TLV is 10 ppm in air. A fume hood should be used at all times when using chloroform.

Cylcohexane is a highly flammable liquid. It may be fatal if swallowed or inhaled, and can cause skin irritation. Use effective fume-removal device. Can react vigorously with strong oxidizing agents.

Dichloromethane (methylene chloride) is toxic and a carcinogen that will emit highly toxic fumes and phosgene gas when heated. The TLV is 100 ppm in air. A fume hood should be used at all times when using methylene chloride.

Diethyl ether is an extremely flammable liquid, and a severe fire and explosion hazard when exposed to heat or flame. It is a central nervous system depressant by inhalation and skin absorption. Store protected from the light. It will form explosive peroxides upon exposure to light. Handle empty containers, particularly those from which ether has evaporated, with extreme caution. Explosive limits in air are 1.85–48%. The TLV is 400 ppm in air. Can react explosively when in contact with chlorine, ozone, lithium aluminum hydride, or strong oxidizing agents. A fume hood should be used at all times when using ethyl ether. Avoid static electricity.

Dimethylformamide is a clear flammable liquid and a strong irritant to skin and tissue. It is toxic by skin absorption. The TLV is 10 ppm in air.

Ethanol (ethyl alcohol) is a clear, colorless, highly flammable liquid. Use effective fume-removal device when heating or evaporating.

Ethyl ether is a highly flammable liquid and a severe fire and explosion hazard when exposed to heat or flame. It is a central nervous system depressant by inhalation and skin absorption. It will form explosive peroxides upon exposure to light. Handle empty containers, particularly those from which ether has evaporated, with extreme caution. Explosive limits in air are 1.85–48%. The TLV is 400 ppm in air. A fume hood should be used at all times when using ethyl ether.

Hexane is a highly flammable solvent and a dangerous fire risk. All work should be performed in a fume hood, with no open flames. The TLV for hexane is 50 ppm in air. OSHA recommends that exposure not exceed 350 ng/M^3 for a time-weighted average. Hexane vapor causes lung irritation and produces neurotoxic effects.

Heptane is a highly flammable liquid and a dangerous fire risk. Vapors may cause lung irritation and may produce neurotoxic effects. A fume hood should be used at all times when using this solvent.

Methanol (methyl alcohol) is flammable liquid, and toxic. Avoid contact with eyes. Avoid breathing vapors. Use effective fume-removal device. Can react vigorously with sodium hydroxide + chloroform, potassium hydroxide + chloroform, and perchloric acid.

Methyl isobutyl ketone (MIBK) is a clear, colorless, and highly flammable liquid and a dangerous fire risk. Explosive limits in air are 1.4–7.5%. Avoid inhalation and ingestion. It is absorbed by the skin. The TLV is 50 ppm in air.

Petroleum ether is the petroleum fraction consisting of aliphatic hydrocarbons in the boiling range 35–60° C. The term *ether* is only figurative, signifying extreme lightness and volatility. It is extremely flammable. The explosive limits in air are 1–6%. Use effective fume-removal device. Avoid static electricity.

Pyridine is a clear liquid with a distinct odor, is highly flammable and a dangerous fire risk. The explosive limits in air are 1.8–12.4%. It is toxic by ingestion and inhalation. The TLV is 5 ppm in air. The danger from crude pyridine is greater than from pure pyridine, the associated homologs and impurities being even more toxic than pyridine itself.

Tetrachloroethylene (perchloroethylene) is a colorless, volatile, nonflammable liquid chlorinated hydrocarbon that will emit toxic fumes of phosgene when exposed to sunlight or flames. It is an irritant to eyes and skin. The TLV is 50 ppm in air.

Tetrahydrofuran is a highly flammable liquid and a dangerous fire risk. The flammable limits in air are 2–11%. It is toxic by ingestion and inhalation. The TLV in air is 200 ppm. It tends to form peroxides upon storage in air.

Toluene is a highly flammable liquid and a dangerous fire risk. Explosive limits in air are 1.27–7%. It is toxic by ingestion, inhalation, and skin absorption. The TLV is 100 ppm in air. A fume hood should be used at all times when using toluene.

Trichloroethane is a synthetic, light-sensitive, volatile, colorless, liquid miscible with many nonpolar organic solvents. It is an irritant to eyes and skin. The TLV is 350 ppm in air.

Xylene is flammable and a dangerous fire risk. The TLV is 100 ppm in air.

CHEMICALS

Chlorine is a poisonous gas. The TLV is 1 ppm in air. It is a strong oxidizing agent and should not be allowed to come in contact with organic materials, hydrogen, powdered metals, and reducing agents. A fume hood should be used at all times when using chlorine.

Gossypol is toxic by ingestion. Avoid contact with particulate matter when working with standards. It is inactivated by heat.

Hydrazine sulfate can cause eye, skin, and mucous membrane irritation and liver and kidney damage. This compound is a known carcinogen in laboratory animals, causing lung and liver tumors in rats. It is a suspected human carcinogen. Precautions should be taken in handling this compound—use gloves, eye protection, and respiratory protection. Avoid the inhalation of dust and powder. Dispose of waste material and waste solutions in a proper and safe manner.

Lead acetate is toxic by ingestion, inhalation, and skin absorption.

Mercury vapors and compounds are extremely toxic and cumulative. Hazardous in contact with ammonia, halogens, and alkali. Regard spills on hot surfaces as extremely hazardous and clean up promptly. Powdered sulfur sprinkled over spilled mercury can assist in cleaning up spills. High degree of personal cleanliness is necessary for persons who use mercury. Handle only in locations that can be readily and completely cleaned up. When mercury evaporation is required, use effective fume-removal device. To avoid environmental contamination, dilute liquid remaining in Kjeldahl digestion flasks to about 300 mL with water, cool to room temperature, and add 50 mL 30% hydrogen peroxide. (If Raney powder method is used, 6 mL of hydrogen peroxide is sufficient.) Warm gently to initiate reaction, let reaction go to completion in warm flask, and separate precipitated mercuric sulfide. Reserve precipitate in closed, labeled container for recovery of mercury or disposal by EPA requirements.

Potassium dichromate is toxic by ingestion and inhalation. There is sufficient evidence in humans for the carcinogenicity of chromium [+6], in particular lung cancer. It is a strong oxidizing agent and a dangerous fire risk in contact with organic chemicals.

Sylon BFT is a powerful silylating reagent, composed of mixing 1 part trimethyl chlorosilane with 99 parts of bistrimethylsilyl-trifluoroacetamide, and should be used only in a properly operating fume hood. This reagent is highly flammable.

tert-Butyl methyl ether is extremely flammable and toxic. Avoid inhalation, ingestion, and eye or skin contact. The TLV is 50 ppm in air. OSHA recommends that exposure not exceed 100 mg/M3 for a time-weighted average. Respiratory irritation, dizziness, and disorientation have been reported. A fume hood should be used at all times when using *tert*-Butyl methyl ether.

Wijs solution, iodine monochloride, causes severe burns, and the vapors can cause lung and eye damage. Use of a fume hood is recommended. Wijs solution without carbon tetrachloride is available commercially.

ADDITIONAL MATERIALS

Castor seeds are poisonous due to the presence of ricin, a highly toxic albumin, and ricinine, a highly toxic alkaloid. Neither pressing nor extraction removes them; hence both hazards remain in the pomace. They also contain an allergenic protein polysaccharide (CB-1A) that is among the most powerful known allergens. It is strongly recommended that workers wear rubber gloves when preparing analytical samples, and that they avoid inhaling any of the dust arising from the castor beans by working near an air exhaust or in a well ventilated laboratory hood.

Fumonisins are hepatotoxic and carcinogenic to rats; effects on humans are not fully known. Wear protective gloves to reduce skin contact with corn extracts. Any laboratory spillages should be washed with a 5% aqueous solution of commercial sodium hypocholorite followed by H_2O. (Dispose of waste solvents according to applicable environmental rules and regulations.)

Mycotoxins should be handled with extreme care because they are highly toxic substances. Perform manipulations under a properly operating fume hood. Take particular precautions, such as the use of a glove box, when toxins are in dry form, because of their electrostatic nature and resulting tendency to disperse in working areas. Swab accidental spills of toxin with 5% NaOCl bleach. Rinse all glassware exposed to toxins with 1% NaOCl bleach solution and then wash thoroughly with warm water.

REFERENCES

Official Methods of Analysis, Association of Official Analytical Chemists, 14th edn., 1984, pp. 1010–1015.

Standard Methods for the Analysis of Oils, Fats and Derivatives, 7th edn., Blackwell Scientific Publications, 1987.

Hawley's Condensed Chemical Dictionary, 11th edn., revised by N. I. Sax and R. J. Lewis, Jr., 1987.