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ASTM Method D 1976-02, Standard Test Method for Elements in Water by
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USPC 231 Heavy Metals

Stepan Company Analytical Method SM 538-A, Low Level Methanol and Ester
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Specifications

Specification PD015 Manufacturing Standard, Methyl Ester, Stepan C-25

Specification RG108 Manufacturing Standard, Esters/Oils, Glycerine USP Kosher

Specification RK102 Manufacturing Standard, Catalysts/Stabilizers, FASCAT 4100

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PROJECT NAME: Stepan® GTC From Methyl Esters
(Filing of Amendment to Self-Affirmed GRAS Petition for Captrin)

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CUSTOMER NAME: Stepan-Internal

REQUESTED BY: Arno Driedger

INDEX TERMS: Medium Chain Triglycerides (MCT), MCT manufacture, methyl ester, C-25 (C8/C10 fatty acid methyl esters), Glycerol Tri(Caprylate/Caprate) (GTC)

LABBOOK REFERENCE: 2593-47, -48, -58; 2720-7, -8, -9, -55, -60; 0022-22-5

SAMPLE REFERENCE/LOT NUMBER: 0022-22-5; 2593-48; 2593-58; 2720-7; 2720-8

DISTRIBUTION: Arno Driedger, Randy Bernhardt, Anne Gariepy, Richard Tenore, Jenifer Heydinger Galante

BACKGROUND:

Stepan Company would like to amend the self-affirmed GRAS petition for Captrin to include an alternate method of manufacture utilizing C8/10 methyl esters (ME). The use of ME provides a potential raw material cost advantage over C8/10 acids. However, using ME could potentially contaminate the final product with trace amounts of catalyst, methanol (a reaction by-product) or ME.

Stepan-Maywood Research & Development Lab prepared five lots of Stepan GTC using C8/10 ME to analyze for trace levels of catalyst, methanol and ME as well as to compare specifications against MCTs made from C8/10 fatty acids.

OBJECTIVE:

Prepare five lots of Stepan GTC using ME and analyze.

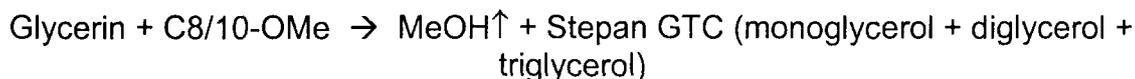
CONCLUSIONS:

Treatment of Stepan GTC (prepared from ME) with activated carbon effectively reduced the catalyst to less than the limits of detection in the final product. Deodorization reduced methanol and ME to less than the limits of detection in the final product.

EXPERIMENTAL DETAILS:

Five lots of Stepan GTC were prepared in the laboratory and in Maywood's pilot plant by transesterifying glycerin with C8/10 ME. An excess of ME was used (110% of theory). The reaction was carried out to a maximum temperature of 220°C in the presence of 500 ppm of a monobutyl tin oxide catalyst to promote reaction. When the reaction began to slow, a slight vacuum (about 20") was pulled to aid in the removal of methanol. When reaction was complete, unreacted ME was removed by vacuum distillation. The product was treated with activated carbon to remove catalyst and reduce color. After filtration, the product was deodorized at 215-220°C at 2-4 mmHg with approximately 2% weight steam to remove methanol and ME. See the process flow diagram in Attachment 1.

Transesterification reaction:



LOT SUMMARY

Five lots of Stepan GTC were prepared and analyzed. Results and the methods used are summarized in Table 1 below. Northfield R&D Analytical Department analyzed the product for residual methanol and ME content using GC/FID. Total heavy metals, tin, arsenic and lead analyses were performed by outside testing facilities using the methods indicated in Table 1.. All other analyses were performed by MW Quality Assurance lab.

RESULTS AND DISCUSSION:

Free Fatty Acid: The amount of free fatty acids in GTC meets the specification for Captrin (0.05 max) except for Lot 2720-7. Slightly less steam was used in the deodorization of this lot than the other 4 lots, all of which met specification; continuing the deodorization of lot 2720-7 would have readily reduced the free fatty acid to within specification. (Deodorization in the plant's commercial unit is much more efficient than in the lab, so achieving the 0.05 max specification will not be a problem.)

Fatty Acid Profile: The fatty acid carbon chain distribution of the product reflects the distribution found in the starting methyl esters. Lot 2593-58 shows that a product with a very low amount of C6 fatty acid can be obtained if the raw material (methyl esters) is low in C6 content. By controlling ME raw material specifications, a desired fatty acid profile in the product can be achieved.

Hydroxyl Value: In order to obtain a product with light color, the reaction is not heated above 220°C. This results in product having a hydroxyl value of 30-60, while M-5 and 1053 have hydroxyl values of 5 or less. Theoretically, this translates to 17-28% weight diglycerides and 1-3% weight monoglycerides in GTC, compared to about 3% weight diglycerides and less than 1% weight monoglycerides in M-5 or 1053. Other commercial MCTs have a hydroxyl value of about 25, which corresponds to 14% weight diglycerides and 1% weight monoglycerides, similar to levels found in GTC. The low levels of mono- and diglycerides found in GTC have no effect on product taste or odor.

GTC meets the same specifications for metals content as Neobee M-5 and 1053. Residual catalyst (tin), methyl ester, and methanol were not detected in GTC.

Table 1
Stepan GTC Summary

Lot Number		2720-8	2720-7	0022-22-5	2593-48	2593-58	Mean	Std. Dev.
Analysis	Method							
Free Fatty Acids, as % oleic	514-O	0.04	0.07	0.03	0.05	0	0.04	0.03
Hydroxyl Value, mg KOH/g	053-O	57	55	37	53	30	46	12
SAP value, mg KOH/g	516-O	322	317	324	323	328	323	4
Moisture, %	501-O	0.04	0.02	0.02	0.02	0.02	0.02	0.01
Color, APHA	006-A	50	69	78	69	27	59	20
Color, Lovibond	006-A	1.4Y, 0.3R	2.0Y, 0.3R	1.9Y, 1.3R	2.0Y, 0.3 R	0.7Y, 0.3R	1.6Y, 0.5R	0.6Y, 0.4R
Odor and Taste	580-O	passes	passes	passes	passes	passes		
GC Fatty Acid Profile, %	499-I							
C6	499-I	1.8	2.2	2.2	2.2	0.0	1.7	1.0
C8	499-I	53.4	62.2	54.4	62.2	54.3	57.3	4.5
C10	499-I	44.2	34.5	42.8	34.5	45.5	40.3	5.4
C12	499-I	0.7	1.1	0.6	1.1	0.0	0.7	0.5
Tin, ppm	ASTM D 1976	<1	<1	<1	<1	<1	<1	0
Arsenic, ppm	ASTM D 1976	<1	<1	<1	<1	<1	<1	0
Lead, ppm	AOAC 999.10	0.01	0.01	0.01	0.01	0.02	0.01	0.004
Total heavy metals, ppm	USP26- NF21S2	<10	<10	<10	<10	<10	<10	0
Methyl esters, ppm**	538-A	n.d.	n.d.	n.d.	n.d.	n.d.		
Methanol, ppm*	538-A	n.d.	n.d.	n.d.	n.d.	n.d.		

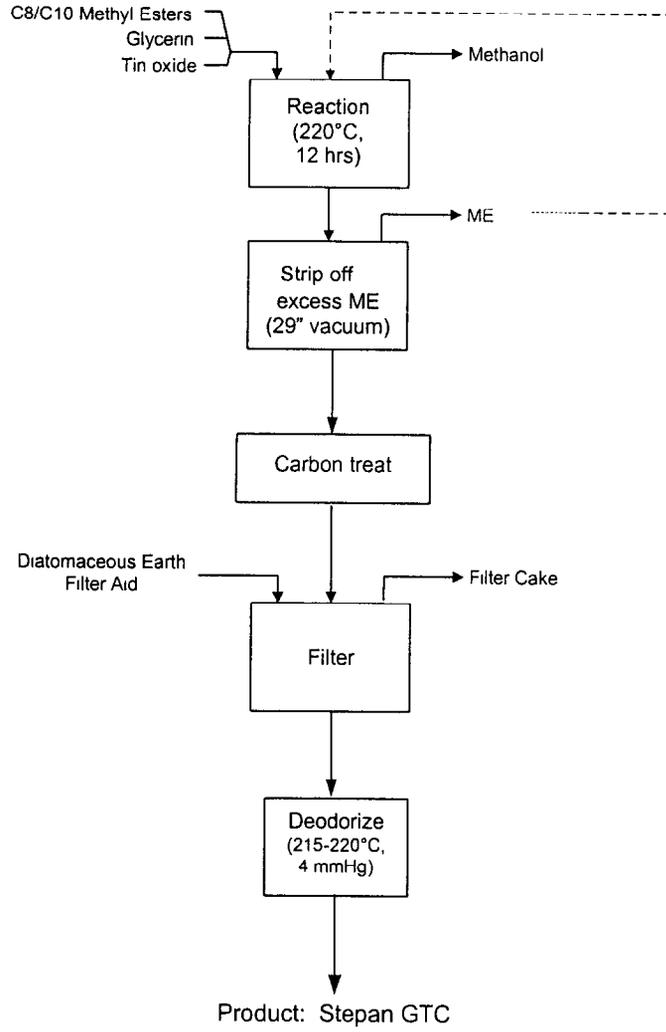
** Limit Of Detection = 0.2 ppm for methanol and 2.5 ppm for total methyl esters

Table 2
Typical Physical Properties of Stepan GTC

Specific Gravity, 25°C	0.95
Smoke Point, °C	182.2
Flash Point, °C	204.4
Freezing Point, °C	-13.7
Viscosity, cPs at 25°C	24.4
Surface Tension, mN/m	25.8

ATTACHMENT 1

Stepan GTC Process Flow Diagram



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9.1.08

AOAC Official Method 999.10
Lead, Cadmium, Zinc, Copper, and Iron in Foods

Atomic Absorption Spectrophotometry
after Microwave Digestion
First Action 1999
NMKL-AOAC Method

[Applicable to determination of Zn, Cu, and Fe in a variety of foods by microwave digestion and flame atomic absorption spectrophotometry (FAAS), and Cd and Pb by microwave digestion and graphite furnace atomic absorption spectroscopy (GFAAS). Method is capable of determining these elements at concentrations above approximately Pb (0.1), Cd (0.01), Zn (4), Cu (0.2), and Fe (7) mg/kg. Method is not applicable to foods with a fat content $\geq 40\%$. Not applicable to milk powder.]

See Table 999.10A for the results of the interlaboratory study supporting acceptance of the method.

Caution Digestion vessels must cool for an appropriate time before opening in order to avoid burns from hot and corrosive vapors. Always gently add acid to water. Maintain safe distance from furnaces equipped with Zeeman background correction when the magnet is on. Consult manufacturer's instructions to determine safe distance, which varies for different instruments. See Appendix B, Laboratory Safety, for safe use of compressed gases, inorganic acids, and atomic absorption spectrometer. For disposal of 4% acetic acid solutions, follow local regulations.

A. Principle

Products are digested with HNO₃ and H₂O₂ under pressure in a closed vessel heated by microwaves. Solution is diluted with H₂O. Pb and Cd are determined by GFAAS. Zn, Cu, and Fe are determined by FAAS.

B. Apparatus

(a) *Atomic absorption spectrophotometer.* With air acetylene burner or nitrous oxide-acetylene burner for flame (FAAS; see Table 999.10B) and a graphite furnace for electrothermal (GFAAS; see Table 999.10C) determinations, with appropriate background (nonatomic) correction.

(b) *Hollow cathode or electrodeless discharge lamps.*—For Pb, Cd, Zn, Cu, and Fe.

(c) *Microwave oven.*—Designed for laboratory use, e.g., MDS-2000, CEM Corp., PO Box 200, Matthews, NC 28106-2000 USA. Microwave oven should be regularly checked for delivered power. If the measured effect does not agree with the specification, adjust the program: Fill a plastic beaker (polypropylene or Teflon) with 1.000 kg water (room temperature) and measure temperature (T_b). Place beaker in microwave oven and heat water at full power for 2 min. Take beaker out of oven, stir water, and measure temperature (T_a). The delivered power in watts:

$$P = 35 \times (T_a - T_b)$$

(d) *Teflon digestion vessels.*—100 mL, withstanding a pressure of at least 1.4 MPa

(e) *Volumetric flasks.*—25 and 1000 mL.

(f) *Funnels.*—Glass or plastic.

(g) *Plastic bottles.*—e.g., Polystyrene bottles with tightly fitting lids, 50–100 mL.

(h) *Drying oven.*— Or equipment for freeze-drying.

All glassware and plasticware should be carefully cleaned and rinsed, e.g., with HNO₃ or HCl, in order to avoid metal contamination.

C. Reagents

Reagents should be of at least analytical reagent grade (p.a.), preferably ultrapure (suprapur) or equivalent.

(a) *Water*—Redistilled or deionized, ≥ 18 M Ω cm.

(b) *Nitric acid.*—65% (w/w).

(c) *Nitric acid.*—0.1M. Dilute 7 mL concentrated HNO₃, (b), with water to 1 L.

(d) *Nitric acid.*—3M. Dilute 200 mL concentrated HNO₃, (b), with water to 1 L.

(e) *Hydrogen peroxide* 30% (w/w).

(f) *Zinc standard solution*—1 mg/mL. Dissolve 1.000 g Zn in 14 mL water + 7 mL nitric acid, (b), in 1 L volumetric flask. Dilute to volume with water [Note: Commercially available standard solutions for AAS (e.g., BDH Chemicals Ltd., Poole, UK) may be used for all metal standard solutions.]

(g) *Copper standard solution.*—1 mg/mL. Dissolve 1.000 g Cu in 7 mL nitric acid, (b), in 1 L volumetric flask. Dilute to volume with water.

(h) *Iron standard solution.*—1 mg/mL. Dissolve 1.000 g Fe in 14 mL water + 7 mL nitric acid, (b), in 1 L volumetric flask. Dilute to volume with water.

(i) *Lead standard solution.*—1 mg/mL. Dissolve 1.000 g Pb in 7 mL HNO₃, (b), in 1 L volumetric flask and dilute to volume with water.

(j) *Cadmium standard solution.*—1 mg/mL. Dissolve 1.000 mg Cd in 14 mL water + 7 mL HNO₃, (b), in 1 L volumetric flask and dilute to volume with water.

(k) *Working standard solutions.*—(1) *For flame analysis.*—Dilute standard, (f)–(j), with 0.1M HNO₃, (c), to a range of standards that covers the concentration of the element to be determined (2) *For graphite furnace analysis.*—Dilute standard solutions, (f)–(j), with 0.1M HNO₃, (c), to a range of standards that covers the linear range of the element to be determined.

D. Procedures

(a) *Cleaning procedure.*—(1) *For glass and plasticware.*—Acid solution: 500 mL concentrated HNO₃, C(b), + 4500 mL deionized water, C(a). Wash first with water and detergent. Rinse with tap water, followed by deionized water, then with acid solution. Finally rinse 4–5 times with deionized water. (2) *For Teflon digestion vessels.*—Rinse with acetone, wash with deionized water, keep vessels covered with 0.1M HNO₃, C(c), for at least 30 min, rinse with deionized water, and let vessels dry

Use separate vessels for different applications, depending on the concentration of metals. If, however, the same digestion vessels are used for heavily contaminated products, e.g., sludge, it may be necessary to use a more severe cleaning procedure, e.g., heating vessels together with concentrated HNO₃, C(b). The instrument manual usually provides detailed instructions for such cleaning procedures

(b) *Pre-treatment.*—If product is to be analyzed fresh, proceed to (d), *Homogenization*. Otherwise, continue at (c), *Drying*

(c) *Drying.*—Dry to constant weight in drying oven at 105°C, or freeze-dry. Freeze-drying is usually preferable because it renders the product less compact and easier to homogenize. If final result is

Table 999.10A. Interlaboratory study results

Metal	Sample	Analyte range,		<i>n</i> ^a	Outliers	<i>s</i> _r	<i>s</i> _R	RSD _r , %	RSD _R , %	<i>r</i>	R
		mg/kg	Mean, mg/kg								
Pb (GFAAS)	Liver	≥0.1	0.130	11	1	0.049	0.055	37	42	0.14	0.15
	Wheat bran		0.155	12	0	0.088	0.091	57	59	0.25	0.26
	Diets ^b		0.394	12	0	0.063	0.098	16	25	0.18	0.27
	Bovine muscle		0.398	10	2		0.086		22		0.24
	Fish		0.48	12	0		0.13		27		0.36
	Mushroom		1.62	12	0		0.26		16		0.73
Cd (GFAAS)	Bovine muscle	≥0.01	0.0124	12	1		0.0034		28		0.0097
	Liver		0.164	13	0	0.025	0.034	15	20	0.070	0.094
	Wheat bran		0.171	11	2	0.0078	0.022	4.6	13	0.022	0.063
	Fish		0.211	12	0		0.035		17		0.099
	Mushroom		0.482	11	2		0.053		11		0.149
	Diets ^b		0.764	12	1	0.050	0.105	6.5	14	0.14	0.294
Zn (FAAS)	Fish	≥4	4.50	12	0		0.41		9.1		1.1
	Milk powder		35.3	14	0		3.3		9.3		9.1
	Diets ^b		47.8	13	1	1.9	2.5	4.0	5.3	5.4	7.1
	Mushroom		56.9	14	0		3.0		5.3		8.4
	Wheat bran		73.5	13	1	2.5	3.5	3.4	4.8	7.1	9.9
	Bovine muscle		147.3	11	3		2.5		1.7		7.0
Cu (FAAS)	Liver		181.9	12	2	2.8	8.8	1.6	4.8	7.9	25
	Fish	≥0.2	0.241	4	0		0.094		3.9		0.26
	Bovine muscle		2.63	6	0		0.17		6.4		0.47
	Wheat bran		10.14	10	1	0.44	0.81	4.3	7.9	1.2	2.3
	Mushroom		37.7	14	0		2.2		5.7		6.0
	Diets ^b		63.42	12	2	0.95	1.9	1.5	3.0	2.7	5.3
Fe (FAAS)	Liver		107.5	14	0	3.3	4.1	3.1	3.8	9.3	12
	Fish	≥7	7.4	9	0		1.3		1.7		3.5
	Bovine muscle		75.0	12	0		8.1		1.1		2.3
	Mushroom		105.5	11	0		7.9		7.5		2.2
	Wheat bran		123.1	12	0	3.9	9.9	3.2	8.1	1.1	2.8
	Diets ^b		303	10	2	1.2	1.8	4.0	5.9	3.3	5.0
	Liver		48.7	12	0	2.7	3.1	5.4	6.4	7.4	8.8

^a *n* = Number of laboratories after outlier elimination. Values for *s*_r, RSD_r, and *r* are only available for duplicate or split level determinations.

^b Simulated diets E and F.

based on fresh weight, weigh test portion before and after drying to obtain water content:

$$H_2O = \frac{W_f - W_d}{W_f} \times 100$$

where H₂O, % = water content of the test portion (%); W_f = weight of the test portion (g); W_d = weight after drying (g).

(d) *Homogenization.* Homogenize products using noncontaminating equipment. Check for leached metals if the apparatus consists of metal parts.

(e) *Digestion.*—Weigh 0.2–0.5 g dry material into digestion vessel. If water-containing materials are used, maximum weight is restricted to 2 g, but dry matter content must never exceed 0.5 g. For example, if product has a water content of 50%, take a maximum of

1 g (= 0.5 g dry matter). If a product has a water content of 95%, take 2 g (<0.5 g dry matter). When unknown products are digested, too much solids may cause the safety membrane in the digestion vessel to rupture.

Add 5 mL HNO₃, C(b), and 2 mL 30% H₂O₂, C(e). Close vessels, place vessels in holder, place vessel holder in microwave oven, and close door. Set oven program according to the parameters given in Table 3 and start program.

The program is valid only when 12 vessels are being digested simultaneously. If fewer are being digested, the remaining vessels must be filled with reagent blank. When a microwave oven other than the one given as an example is used, it may be necessary to use a slightly different time/power program.

Remove digestion vessels from microwave oven and let cool thoroughly before opening them. Open vessel and rinse down lid and

Table 999.10B. Instrumental parameters for FAAS

Metal	Flame type	Wavelength, nm
Zn	Air-acetylene, oxidizing	213.9
Cu	Air-acetylene, oxidizing	324.7
Fe	Air-acetylene, oxidizing	248.3
Fe	N ₂ O-acetylene, oxidizing	248.3

walls into container. Transfer solution to 25 mL volumetric flask and dilute to mark with deionized water. Then, transfer solution to plastic container. Treat blanks in the same way as tests. One blank should be included in every set.

(f) *Dilution*.—If test solution needs to be further diluted (due to high metal concentrations), dilute with 3M HNO₃, C(d), in order to maintain same acid concentration prior to metal determination, (g)

High acid concentration is environmentally undesirable and may depress the analytical signal. Reduce acid strength by diluting the test solution 1/2 with 0.1M nitric acid and standard solutions 1/2 with 3M nitric acid. The tests and standards are thereby brought to the same acid concentration. Matching of acid concentrations is important when a calibration curve is used.

(g) *Atomic absorption spectrophotometry*.—Use of flame or graphite furnace technique is determined by the concentration of the metal to be determined. Flame technique should be used as far as possible, since this technique is less sensitive to interference than the GFAAS. The most appropriate wavelength, gas mixture/temperature program, and other instrumental parameters for each metal are found in the manual provided with the instrument. Always use background correction.

Measurements must be within the linear range when the method of standard addition is used. A standard addition curve consists of at least 3 points, of which at least 2 are standards. The concentration of the highest standard should be 3–5 times the concentration in the test solution. The lower standard should have a concentration approximately half of the highest standard. A simplified version of the method of standard addition is to use a matrix-matched standard curve, which is applicable to products with the same matrix: The test and standard solutions are mixed and used to make a standard addition curve. This curve is then parallel transferred to origin and is used as the standard curve for the tests that followed and that have been diluted in the same proportions. The matrix-matched standard curve and the test solutions will thus have the same matrix concentration. On most modern instruments, this function is included in the software.

(1) *Flame technique*.—The concentration of Zn, Cu, and Fe are usually at levels suitable for determination by FAAS. When calibration curve is to be used, standards and test solutions must have the same acid concentration.

Table 999.10C. Instrumental parameters for GFAAS

Metal	Wavelength, nm	Temperature (°C)/ramp-hold (s)		Cleaning out step (°C)
		Ashing step	Atomization step	
Pb	283.3	650/15-10	1900/0-4	2500
Cd	228.8	350/15-10	1200/0-4	2500

Table 999.10D. Parameters for microwave oven program

Step	Power (watts)	Duration (min)
1	250	3
2	630	5
3	500	22
4	0	15

Since Fe may be strongly affected by interferences from the matrix, use either the method of standard addition or matrix-matched standards. When experiencing severe interferences, an oxidizing nitrous oxide acetylene flame may be an alternative

(2) *Graphite furnace technique*.—This technique is generally required for determination of Pb and Cd in foods. Use pyrolytically coated tubes with platforms. Since the method results in a fairly large dilution of the analyte, it may frequently be needed also for the determination of, e.g., Cu. The method of standard addition or matrix-matched standards should always be used unless shown to be unnecessary (i.e., no significant difference between the slopes of calibration curves of pure working standard and standard addition curves of the test product). Measurements must be made in the linear range when the method of addition is used.

Program the autosampler to deliver a volume that gives as large an absorbance as possible within the linear range and producing a background absorbance not larger than approximately 0.5 absorbance units. Multiple injection may enhance the absorbance at very low concentrations. Evaluate each new matrix by means of ash- and atomization-curves in order to optimize the graphite furnace parameters.

E. Calculations and Evaluation of Results

Calculate the concentration (C) of metal in the test sample according to the formula:

$$C = \frac{(a - b)df \times 25}{m}$$

where C = concentration in the test sample (mg/kg); a = concentration in the test solutions (mg/L); df = dilution factor; b = mean concentration in the blank solutions (mg/L); m = weight of the test portion (g)

If (a - b) is lower than the detection limit, DL, then (a - b) is replaced by DL for calculation of the limit of detection in the test sample.

If the test solution has been diluted, the dilution factor (df) has to be taken into account. If the test portion was dried and the result should be based on fresh weight, correct according to the following.

$$C_{FW} = C \times \frac{100 - H_2O\%}{100}$$

where C_{FW} = concentration in the test portion corrected to fresh weight (mg/kg); H₂O% = the water content of the test portion (%).

When running replicates, the average of the results should be given with 3 significant figures.

Detection limit.—The DL for each metal is calculated as DL = 3 × standard deviation of the mean of the blank determinations (n = ≥20). A large number of blanks must be analyzed before DL can be established. A DL is not static and will need to be re-evaluated from time to time in accordance with changes in the blank levels.

Reference: *J. AOAC Int.* 83, 1189(2000).



Standard Test Method for Elements in Water by Inductively-Coupled Argon Plasma Atomic Emission Spectroscopy¹

This standard is issued under the fixed designation D 1976, the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the determination of dissolved, total-recoverable, or total elements in drinking water, surface water, domestic, or industrial wastewaters.^{2,3}

1.2 It is the user's responsibility to ensure the validity of the test method for waters of untested matrices.

1.3 Table 1 lists elements for which this test method applies, with recommended wavelengths and typical estimated instrumental detection limits using conventional pneumatic nebulization.⁴ Actual working detection limits are sample dependent and as the sample matrix varies, these detection limits may also vary. In time, other elements may be added as more information becomes available and as required.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements, see Note 2 and Section 9.

2. Referenced Documents

2.1 ASTM Standards

- D 1066 Practice for Sampling Steam⁵
- D 1129 Terminology Relating to Water⁵
- D 1192 Specification for Equipment for Sampling Water and Steam in Closed Conduits⁵
- D 1193 Specification for Reagent Water⁵
- D 2777 Practice for Determination of Precision and Bias of

¹ This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.05 on Inorganic Constituents in Water.

Current edition approved May 10, 2002. Published June 2002. Originally published as D 1976-91. Last previous edition D 1976-96.

² The detailed report of EPA Method Study 27, Method 200.7 is available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA. A summary of the project is available from the U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, OH.

³ Fishman, M. J. and Friedman, L., "Methods for Determination of Inorganic Substances in Water and Fluvial Sediments", *U.S. Geological Survey Techniques of Water-Resources Investigations*, Book 5, Chapter, Open File Report 85-495, 1985, p. 659-671.

⁴ Winge, R. K., Fassel, V. A., Peterson, V. J., and Floyd, M. A., "Inductively Coupled Plasma-Atomic Emission Spectroscopy," *An Atlas of Spectral Information*, Elsevier Science Publishing Co., Inc., New York, NY, 1985.

⁵ *Annual Book of ASTM Standards*, Vol. 11.01.

TABLE 1 Suggested Wavelengths and Estimated Detection Limits⁴

Element	Wavelength, nm ^A	Estimated detection limit, μg/L ^B
Aluminum	308.215	45
Arsenic	193.696	53
Antimony	206.833	32
Beryllium	313.042	0.3
Boron	249.773	5
Cadmium	226.502	4
Chromium	267.716	7
Cobalt	228.616	7
Copper	324.754	6
Iron	259.940	7
Lead	220.353	42
Magnesium	279.079	30
Manganese	257.610	2
Molybdenum	202.030	8
Nickel	231.604	15
Selenium	196.026	75
Silver	328.068	7
Thallium	190.864	40
Vanadium	292.402	8
Zinc	213.856	2

^A The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see 6.1.1).

^B The estimated detection limits as shown are taken from Winge, Fassel, *et al.*⁴ They are given as a guide for approximate detection limits for the listed wavelengths. The actual test method instrumental detection limits are sample-dependent and may vary as the sample matrix varies (see 3.1.4).

- Applicable Test Methods of Committee D-19 on Water⁵
- D3370 Practices for Sampling Water from Closed Conduits⁵
- D 4841 Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents⁵
- D 5810 Guide for Spiking into Aqueous Samples³
- D 5847 Practice for the Writing Quality Control Specifications for Standard Test Methods for Water Analysis⁶

3. Terminology

3.1 Definitions of Terms Specific to This Standard.

3.1.1 *calibration blank, n*—a volume of water containing the same acid matrix as the calibration standards (see 11.1).

3.1.2 *calibration standards, n*—a series of known standard solutions used by the analyst for calibration of the instrument (preparation of the analytical curve) (see 8.11).

⁶ *Annual Book of ASTM Standards*, Vol. 11.02.

3.1.3 *dissolved, adj*—those elements that will pass through a 0.45 μm membrane filter.

3.1.4 *instrumental detection limit, n*—the concentration equivalent to a signal, due to the analyte, that is equal to three times the standard deviation of a series of ten replicate measures of a reagent blank signal at the same wavelength.

3.1.5 *reagent blank, n*—a volume of water containing the same matrix as the calibration standards, carried through the entire analytical procedure.

3.1.6 *total, n*—the concentration determined on an unfiltered sample following vigorous digestion (see 12.3).

3.1.7 *total-recoverable, adj*—a term relating to forms of each element that are determinable by the digestion method that is included in this procedure (see 12.2).

3.1.8 *laboratory control sample, n*—a solution with the certified concentration(s) of the analytes

3.2 *Definitions*—For definitions of other terms used in this test method, refer to Terminology D 1129.

4. Summary of Test Method

4.1 Elements are determined, either sequentially or simultaneously, by inductively-coupled argon plasma optical emission spectroscopy.

4.2 A background correction technique may be used to compensate for variable background contribution from high concentrations of major and trace elements.

5. Significance and Use

5.1 This test method is useful for the determination of element concentrations in many natural waters and wastewaters. It has the capability for the simultaneous determination of up to 20 elements. High analysis sensitivity can be achieved for some elements that are difficult to determine by other techniques

6. Interferences

6.1 Several types of interference effects may contribute to inaccuracies in the determination of trace elements. These interferences can be summarized as follows

6.1.1 Spectral interferences can be categorized as (1) overlap of a spectral line from another element; (2) unresolved overlap of molecular band spectra; (3) background contribution from continuous or recombination phenomena; and (4) background contribution from stray light from line emission of high concentration elements.

6.1.1.1 The effects described in 6.1.1 can be compensated for by utilizing a computer correction of the raw data, requiring the monitoring and measurement of the interfering element. The second effect may require selection of an alternate wavelength. The third and fourth effects can usually be compensated for by a background correction adjacent to the analyte line.

6.1.1.2 Table 2 lists some interference effects for the recommended wavelengths given in Table 1. The data in Table 2 are intended for use only as a rudimentary guide for the indication of potential spectral interferences. For this purpose, linear relations between concentration and intensity for the analytes and the interferents can be assumed

6.1.1.3 Only those interferents listed in Table 2 were investigated. The blank spaces in Table 2 indicate that measurable interferences were not observed for the interferent concentrations listed in Table 3. Generally, interferences were considered as discernible if the interferent produced interference peaks or background shifts that corresponded to 2 to 5 % of the peaks generated by the analyte concentrations also listed in Table 3

6.1.2 Physical interferences are generally considered to be effects associated with the sample nebulization and transport processes. Such properties as change in viscosity and surface tension can cause significant inaccuracies, especially in

TABLE 2 Analyte Concentration Equivalents, mg/L, Arising from Interferents at the 100 mg/L Level^A

Analyte	Wavelength, nm	Interferent									
		Al	Ca	Cr	Cu	Fe	Mg	Mn	Ni	Ti	V
Aluminum	308.215							0.21			1.4
Antimony	206.833	0.47	..	2.9		0.08				0.25	0.45
Arsenic	193.696	1.3		0.44							1.1
Barium	455.403										
Beryllium	313.042									0.04	0.05
Boron	249.773	0.04				0.32					
Cadmium	226.502			..		0.03			0.02		
Calcium	317.933			0.08		0.01	0.01	0.04		0.03	0.03
Chromium	267.716					0.003		0.04			0.04
Cobalt	228.616			0.03		0.005			0.03	0.15	
Copper	324.754			0.003				0.05	0.02
Iron	259.940						0.12	0.12			
Lead	220.353	0.17									
Magnesium	279.079		0.02	0.11		0.13	0.002	0.25		0.07	0.12
Manganese	257.610	0.005		0.01		0.002					
Molybdenum	202.030	0.05				0.03					
Nickel	231.604										
Selenium	196.026	0.23				0.09					
Silicon	288.158			0.07							0.01
Sodium	588.995									0.08	
Thallium	190.864	0.30									
Vanadium	292.402		..	0.05		0.005				0.02	
Zinc	213.856				0.14					0.29	

^A See Table 3 for concentrations used

TABLE 3 Interferent and Analyte Elemental Concentrations^A

Analytes	mg/L	Interferents	mg/L
Al	10	Al	1 000
As	10	Ca	1 000
B	10	Cr	200
Ba	1	Cu	200
Be	1	Fe	1 000
Ca	1	Mg	1 000
Cd	10	Mn	200
Co	1	Ni	200
Cr	1	Ti	200
Cu	1	V	200
Fe	1		
Mg	1		
Mn	1		
Na	10		
Ni	10		
Pb	10		
Sb	10		
Se	10		
Si	1		
Ti	10		
V	1		
Zn	10		

^A This table indicates concentrations used for interference measurements in Table 2

samples that may contain high dissolved solids or acid concentrations, or both. The use of a peristaltic pump may lessen these interferences. If these types of interferences are operative, they must be reduced by dilution of these samples or utilization of standard addition techniques, or both.

6.1.2.1 Salt buildup at the tip of the nebulizer is another problem that can occur from high dissolved solids. This salt buildup affects aerosol flow rate that can cause instrumental drift. To control this problem, wet the argon prior to nebulization, use a tip washer, or dilute the sample.

NOTE 1—Periodic inspection and cleaning of the nebulizer and torch components are highly recommended.

6.1.2.2 Reports indicate that better control of the argon flow rate improves instrument performance. This control of the argon flow rate can be accomplished with the use of mass flow controllers.

6.1.3 Chemical interferences are characterized by molecular compound formation, ionization effects, and solute vaporization effects. Normally these effects are not pronounced with the ICP technique; however, if observed, they can be minimized by careful selection of operating conditions (incident power, plasma observation position, and so forth), by buffering the sample, by matrix matching, and by standard addition procedures. These types of interferences can be highly dependent on matrix type and the specific analyte.

7. Apparatus

7.1 See the manufacturer's instruction manual for installation and operation of inductively-coupled argon plasma spectrometers.

8. Reagents and Materials

8.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that reagents shall conform to the specifications of the Committee

on Analytical Reagents of the American Chemical Society⁷. The high sensitivity of inductively-coupled argon plasma atomic emission spectrometry may require reagents of higher purity. Stock standard solutions are prepared from high purity metals, oxides, or nonhydroscopic reagent grade salts using Types I, II, and III reagent water, and ultrapure acids. Other grades may be used, provided it is first ascertained that the reagent is of sufficient purity to permit its use without lessening the accuracy of the determination.

8.2 *Purity of Water*—Unless otherwise indicated, reference to water shall be understood to mean reagent water conforming to Type I, II, or III of Specification D 1193. It is the analyst's responsibility to assure that water is free of interferences. Other reagent water types may be used provided it is first ascertained that the water is of sufficiently high purity to permit its use without adversely affecting the precision and bias of the test method. Type II water was specified at the time of round robin testing of this test method.

8.3 *Aqua Regia*—Mix three parts hydrochloric acid (sp gr 1.19) and one part concentrated nitric acid (sp gr 1.42) just before use.

NOTE 2—Exercise caution when mixing this reagent.

8.4 *Argon*—Welding grade equivalent or better.

8.5 *Hydrochloric Acid* (sp gr 1.19)—Concentrated hydrochloric acid, ultrapure or equivalent.

8.6 *Hydrochloric Acid* (1 + 1)—Add 1 vol of hydrochloric acid (sp gr 1.19) to 1 vol of water.

8.7 *Nitric Acid* (sp gr 1.42)—Concentrated nitric acid, ultrapure or equivalent.

8.8 *Nitric Acid* (1 + 1)—Add 1 vol of nitric acid (sp gr 1.42) to 1 vol of water.

8.9 *Nitric Acid* (1 + 499)—Add 1 vol of nitric acid (sp gr 1.42) to 499 vol of water.

8.10 *Stock Solutions*—Preparation of stock solutions for each element is listed in Table 4.

8.11 *Mixed Calibration Standard Solutions*—Prepare mixed calibration standard solutions by combining appropriate volumes of the stock solutions in volumetric flasks (see Note 3). Prior to preparing mixed standards, each stock solution should be analyzed separately to determine possible spectral interference or the presence of impurities. Care should be taken when preparing the mixed standards to ensure the elements are compatible and stable.

NOTE 3—Mixed calibration standards will vary depending on the number of elements being determined. An example of mixed calibration standards for the simultaneous determination of 20 elements is as follows:

Mixed Standard Solution I—manganese, beryllium, cadmium, lead, and zinc
 Mixed Standard Solution II—copper, vanadium, iron, and cobalt
 Mixed Standard Solution III—molybdenum, arsenic, and selenium
 Mixed Standard Solution IV—aluminum, chromium, and nickel
 Mixed Standard Solution V—antimony, boron, magnesium, silver, and thallium

⁷ *Reagent Chemicals, American Chemical Society Specifications*. American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd, Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.

TABLE 4 Preparation of Metal Stock Solutions^{A,B}

Element (Compound)	Weight, g	Solvent
Al	0.1000	HCl (1 + 1)
Sb	0.1000	Aqua regia
As ₂ O ₃ ^C	0.1320	Water + 0.4 g NaOH
Be	0.1000	Aqua regia
H ₃ BO ₃	0.5716	Water
Cd	0.1000	HNO ₃ (sp gr 1.42)
Cr	0.1000	HCl (1 + 1)
Co	0.1000	HNO ₃ (1 + 1)
Cu	0.1000	HNO ₃ (1 + 1)
Fe	0.1000	HNO ₃ (sp gr 1.42)
Pb	0.1000	HNO ₃ (sp gr 1.42)
Mg	0.1000	HNO ₃ (1 + 1)
Mn	0.1000	HNO ₃ (1 + 1)
Ni	0.1000	HNO ₃ (sp gr 1.42)
(NH ₄) ₂ MoO ₄	0.2043	Water
Na ₂ SeO ₄ ^D	0.2393	Water
Ag	0.1000	HNO ₃ (sp gr 1.42)
TiNO ₃	0.1303	Water
NH ₄ VO ₃	0.2297	HNO ₃ (1 + 1)
Zn	0.1000	HNO ₃ (1 + 1)

^A Metal stock solutions, 1.00 mL = 100 µg of metal. Dissolve the listed weights of each compound or metal in 20 mL of specified solvent and dilute to 1 L. The metals may require heat to increase rate of dissolution.

^B Where water is used as the solvent, acidify with 10 mL of HNO₃(sp gr 1.42) and dilute to 1 L. See Section 8 for concentration of acids. Commercially available standards may be used. Alternative salts or oxides may also be used.

^C Add 2 mL of HNO₃(sp gr 1.42) and dilute to 1 L.

^D Add 1 mL of HNO₃(sp gr 1.42) and dilute to 1 L.

8.12 *Reagent Blank*—This must contain all the reagents and be the same volume as used in the processing of the samples. The reagent blank must be carried through the complete procedure and contain the same acid concentration in the final solution as the sample solution used for analysis.

9. Hazards

9.1 The toxicity or carcinogenicity of each reagent used in this test method has not been precisely defined; however, each chemical should be treated as a potential health hazard. Adequate precautions should be taken to minimize personnel exposure to chemicals used in this procedure.

10. Sampling

10.1 Collect the samples in accordance with Practice D 1066 or D 3370 or Specification D 1192, as applicable.

10.2 Preserve the samples by immediately adding nitric acid to adjust the pH to 2 at the time of collection. Normally, 2 mL of HNO₃ is required per litre of sample. If only dissolved elements are to be determined, filter the sample through a 0.45-µm membrane filter before acidification (see Note 4). The holding time for the sample may be calculated in accordance with Practice D 4841.

NOTE 4 Depending on the manufacturer, some filters have been found to be contaminated to various degrees with heavy metals. Care should be exercised in selecting a source for these filters. It is good practice to wash the filters with dilute nitric acid and a small portion of the sample before filtering.

11. Calibration and Standardization

11.1 Calibrate the instrument over a suitable concentration range for the elements chosen by atomizing the calibration blank and mixed standard solutions and recording their concentrations and signal intensities. Because the precision and

bias for this test method was obtained using a two-point calibration, it is recommended that the instrument be calibrated using this procedure as outlined in the test method. Multiple-point calibration standards may be used, but it is the user's responsibility to ensure the validity of the test method. Regardless of the calibration procedure used, appropriate QC is required to verify the calibration curve at the anticipated concentration range(s) before proceeding to the sample analysis. It is recommended that the calibration blank and standard be matrix matched with the same acid concentration contained in the samples.

12. Procedure

12.1 To determine dissolved elements proceed with 12.4.

12.2 When determining total-recoverable elements, choose a volume of a well mixed, acid-preserved sample appropriate for the expected level of elements.

12.2.1 Transfer the sample to a beaker and add 2 mL of HNO₃(1 + 1) and 10 mL of HCl (1 + 1) and heat on a steam bath or hot plate until the volume has been reduced to near 25 mL, making certain the sample does not boil. Cool the sample, and if necessary filter or let insoluble material settle to avoid clogging of the nebulizer. Adjust to the original sample volume. To determine total-recoverable elements, proceed with 12.4.

12.3 When determining total elements, choose a volume of well mixed, acid-preserved sample appropriate for the expected level of elements.

12.3.1 Transfer the sample to a beaker. Add 3 mL of HNO₃(sp gr 1.42). Place the beaker on a hot plate and cautiously evaporate to near dryness, making certain that the sample does not boil and that no area of the bottom of the beaker is allowed to go dry. Cool the beaker and add 5 mL of HNO₃(sp gr 1.42). Cover the beaker with a watch glass and return it to the hot plate. Increase the temperature of the hot plate so a gentle reflux action occurs. Continue heating, adding additional acid as necessary, until the digestion is complete (generally indicated when the digestate is light in color or does not change in appearance with continued refluxing). Again, evaporate to near dryness and cool the beaker. Add 10 mL of HCl (1 + 1) and 15 mL of water per 100 mL of final solution and warm the beaker gently for 15 min to dissolve any precipitate or residue resulting from evaporation. Allow the sample to cool, wash the beaker walls and watch glass with water, and if necessary, filter or let insoluble material settle to avoid clogging the nebulizer. Adjust to the original sample volume. To determine total elements, proceed with 12.4.

12.4 Atomize each solution to record its emission intensity or concentration. A sample rinse of HNO₃(1 + 499) is recommended between samples.

13. Calculation

13.1 Subtract reagent blanks (see 8.12) from all samples. This subtraction is particularly important for digested samples requiring large quantities of acids to complete the digestion.

13.2 If dilutions are required, apply the appropriate dilution factor to sample values.

13.3 Report results in the calibration concentration units.

TABLE 5 Regression Equations for Bias and Precision, µg/L, Reagent Water versus Surface Water (Aluminum, Antimony, Arsenic, Beryllium)

NOTE 1— X = mean recovery, C = true value for the concentration.

Water Type	Aluminum	Antimony	Arsenic	Beryllium
Total Digestion				
Applicable concentration range	(83 to 1434)	(411 to 1406)	(83 to 943)	(17 to 76)
Reagent water, hard				
Single-analyst precision	$S_o = 0.05X + 3.72$	$S_o = 0.23X - 50.17$	$S_o = 0.07X + 8.28$	$S_o = 0.02X + 0.18$
Overall precision	$S_i = 0.07X + 9.34$	$S_i = 0.21X - 24.02$	$S_i = 0.11X + 2.96$	$S_i = 0.02X + 0.91$
Bias	$X = 0.91C + 6.62$	$X = 0.74C + 2.27$	$X = 1.03C - 12.03$	$X = 1.02C - 1.92$
Surface water, hard				
Single-analyst precision	$S_o = 0.00X + 40.75$	$S_o = 0.11X - 0.14$	$S_o = 0.05X + 7.79$	$S_o = 0.00X + 0.85$
Overall precision	$S_i = 0.10X + 67.23$	$S_i = 0.07X + 35.71$	$S_i = 0.10X + 10.55$	$S_i = 0.09X - 0.47$
Bias	$X = 0.98C + 90.54$	$X = 0.88C - 55.19$	$X = 1.00C - 16.02$	$X = 1.00C - 0.89$
Total-Recoverable Digestion				
Applicable concentration range	(83 to 1434)	(411 to 1406)	(83 to 943)	(17 to 76)
Reagent water, soft				
Single-analyst precision	$S_o = 0.05X + 25.05$	$S_o = 0.06X + 7.85$	$S_o = 0.07X + 6.12$	$S_o = 0.04X + 0.14$
Overall precision	$S_i = 0.10X + 28.72$	$S_i = 0.05X + 20.10$	$S_i = 0.12X + 2.99$	$S_i = 0.07X - 0.47$
Bias	$X = 0.93C + 28.40$	$X = 0.92C - 22.46$	$X = 1.01C - 2.08$	$X = 1.03C - 0.73$
Reagent water, soft				
Single-analyst precision	$S_o = 0.01X + 34.72$	$S_o = 0.06X + 0.97$	$S_o = 0.05X + 9.29$	$S_o = 0.02X + 0.43$
Overall precision	$S_i = 0.10X + 74.75$	$S_i = 0.07X + 14.28$	$S_i = 0.11X + 1.82$	$S_i = 0.01X + 15.4$
Bias	$X = 1.02C + 40.42$	$X = 0.95C - 34.50$	$X = 1.06C - 7.00$	$X = 1.04C - 2.08$

14. Precision and Bias ⁸

14.1 The precision and bias data for this test method are based on an interlaboratory study conducted by the U.S. Environmental Protection Agency.²

14.2 The test design of the study meets the requirements of Practice D 2777 – 86 for elements listed in this test method. Barium, calcium, lithium, potassium, silica, and sodium did not meet the requirements of Practice D 2777 – 86 and are outlined in Appendix X1.

14.2.1 The test design is based on a form of the analysis of variance applying the approach and methods of the Youden Unit block design. In the Youden nonreplicate approach to determining the precision and bias of the analytical method, pairs of samples of similar but different concentrations are analyzed. The key in the Youden approach is to estimate precision from analyses of Youden pairs rather than through replicate analyses. In the referenced study, five Youden pairs of spike materials were prepared (D 5810). Six water types were included. Only the data from reagent water and surface water are presented here. Each water type was spiked with three of the five Youden pairs with the exception of reagent water, which was spiked with all five Youden pairs. Each water sample was prepared for analysis by both a total and a total-recoverable digestion procedure. A total of twelve laboratories participated in the study.

14.2.2 Type II water was specified for this round robin

14.2.3 Twenty-seven different elements were included in the study and individual measurements of precision and bias were developed for each. Bias was related to mean recovery of the analyte. The equation used to summarize accuracy data over concentration for each water type/digestion type/element was.

$$X = a + b \times C$$

where:

X = mean recovery of the element,

a = intercept,

b = slope, and

C = concentration level of the element.

14.2.4 The precision of the test method has been related to the overall and single analyst variation of the test method. Equations used to summarize precision data over concentration for each water type/digestion type/element were:

$$S_i = d + e \times X$$

where:

S_i = overall standard deviation, and

$$S_o = f + g \times X$$

where:

S_o = single analyst standard deviation,

f = intercept, and

g = slope.

The results for reagent water and surface water for these equations are presented in Tables 5-9.

14.2.5 These data may not apply to waters of other matrices; therefore, it is the responsibility of the analyst to ensure the validity of the test method in a particular matrix. Matrix effects and potential contamination encountered in this study can be found in Appendix X2.

14.3 Precision and bias for this test method conforms to Practice D 2777-77, which was in place at the time of collaborative testing. Under the allowances made in 1.4 of D 2777-98, these precision and bias data do meet existing requirements for interlaboratory studies of Committee D19 test methods.

⁸ Supporting data are available from ASTM International Headquarters. Request RR D-19-1144

TABLE 6 Regression Equations for Bias and Precision, µg/L, Reagent Water versus Surface Water (Boron, Cadmium, Chromium, Cobalt)

 NOTE 1— X = mean recovery; C = true value for the concentration.

Water Type	Boron	Cadmium	Chromium	Cobalt
Total Digestion				
Applicable concentration range	(330 to 1179)	(18 to 776)	(25 to 470)	(58 to 843)
Reagent water, hard				
Single-analyst precision	$S_o = -0.02X + 62.67$	$S_o = 0.02X + 1.49$	$S_o = 0.01X + 3.74$	$S_o = 0.04X + 1.17$
Overall precision	$S_t = -0.02X + 75.99$	$S_t = 0.07X + 1.40$	$S_t = 0.02X + 4.72$	$S_t = 0.06X + 0.21$
Bias	$X = 0.97C - 39.09$	$X = 0.98C + 0.20$	$X = 0.98C - 0.96$	$X = 0.93C - 4.34$
Surface water, hard				
Single-analyst precision	$S_o = 0.02X + 73.05$	$S_o = 0.04X + 0.23$	$S_o = 0.01X + 2.83$	$S_o = 0.03X + 1.45$
Overall precision	$S_t = 0.11X + 38.83$	$S_t = 0.08X + 1.94$	$S_t = 0.07X + 2.77$	$S_t = 0.03X - 4.30$
Bias	$X = 0.94C + 0.99$	$X = 1.00C + 0.28$	$X = 0.98C + 2.18$	$X = 0.94C - 2.97$
Total-Recoverable Digestion				
Applicable concentration range	(330 to 1179)	(18 to 776)	(25 to 470)	(58 to 843)
Reagent water, soft				
Single-analyst precision	$S_o = 0.05X + 53.98$	$S_o = 0.03X + 1.07$	$S_o = 0.04X + 3.56$	$S_o = 0.05X - 0.22$
Overall precision	$S_t = 0.07X + 73.55$	$S_t = 0.05X + 1.36$	$S_t = 0.07X + 2.55$	$S_t = 0.06X + 2.29$
Bias	$X = 1.10C - 77.26$	$X = 1.01C + 0.45$	$X = 1.01C - 1.85$	$X = 0.93C - 1.01$
Reagent water, soft				
Single-analyst precision	$S_o = -0.02X + 62.90$	$S_o = 0.03X + 0.18$	$S_o = 0.02X + 5.18$	$S_o = 0.02X + 4.80$
Overall precision	$S_t = 0.06X + 32.16$	$S_t = 0.09X + 0.17$	$S_t = 0.05X + 6.83$	$S_t = 0.05X + 4.89$
Bias	$X = 1.07C - 2.83$	$X = 1.02C - 0.58$	$X = 0.98C + 0.30$	$X = 0.93C - 0.28$

TABLE 7 Regression Equations for Bias and Precision, µg/L, Reagent Water versus Surface Water (Copper, Iron, Lead, Magnesium)

 NOTE 1— X = mean recovery, C = true value for the concentration.

Water Type	Copper	Iron	Lead	Magnesium
Total Digestion				
Applicable concentration range	(17 to 189)	(74 to 2340)	(85 to 943)	(73 to 4623)
Reagent water, hard				
Single-analyst precision	$S_o = 0.02X + 2.02$	$S_o = 0.04X + 2.34$	$S_o = 0.03X + 4.56$	$S_o = 0.03X + 0.24$
Overall precision	$S_t = 0.02X + 3.66$	$S_t = 0.04X + 17.09$	$S_t = 0.01X + 18.87$	$S_t = 0.04X + 17.24$
Bias	$X = 0.94C - 1.23$	$X = 0.99C - 11.50$	$X = 0.97C - 3.09$	$X = 1.01C - 5.94$
Surface water, hard				
Single-analyst precision	$S_o = 0.00X + 4.40$	$S_o = 0.11X + 3.13$	$S_o = 0.02X + 7.44$	$S_o = 0.02X + 58.13$
Overall precision	$S_t = 0.04X + 3.81$	$S_t = 0.14X + 26.28$	$S_t = 0.05X + 8.36$	$S_t = 0.10X + 41.28$
Bias	$X = 0.98C - 1.56$	$X = 0.98C + 34.94$	$X = 0.98C - 4.58$	$X = 1.03C + 84.36$
Total-Recoverable Digestion				
Applicable concentration range	(17 to 189)	(74 to 2340)	(85 to 943)	(73 to 4623)
Reagent water, soft				
Single-analyst precision	$S_o = 0.03X + 1.73$	$S_o = 0.08X + 10.52$	$S_o = 0.05X + 4.18$	$S_o = 0.05X - 0.47$
Overall precision	$S_t = 0.05X + 2.55$	$S_t = 0.10X + 13.84$	$S_t = 0.10X + 3.09$	$S_t = 0.08X + 6.78$
Bias	$X = 0.98C - 4.68$	$X = 1.03C - 3.35$	$X = 0.99C + 11.21$	$X = 1.00C - 3.61$
Reagent water, soft				
Single-analyst precision	$S_o = 0.01X + 4.43$	$S_o = 0.01X + 53.15$	$S_o = 0.02X + 6.38$	$S_o = 0.15X + 0.24$
Overall precision	$S_t = 0.03X + 4.95$	$S_t = 0.05X + 51.00$	$S_t = 0.06X + 8.77$	$S_t = 0.19X + 109.84$
Bias	$X = 0.98C - 1.38$	$X = 1.01C + 10.13$	$X = 0.98C + 3.92$	$X = 0.96C + 104.38$

15. Quality Control (QC)

15.1 The following quality control information is recommended for measuring elements in water by Inductively-Coupled Argon Plasma Atomic Emission Spectroscopy.

15.2 The instrument shall be calibrated using a minimum of four calibration standards and a calibration blank. The calibration correlation coefficient shall be equal to or greater than 0.990. In addition to the initial calibration blank, a calibration blank shall be analyzed at the end of the batch run to ensure contamination was not a problem during the batch analysis.

15.3 An instrument check standard shall be analyzed at a minimum frequency of 10 % throughout the batch analysis. The value of the instrument check standard shall fall between 80 % and 120 % of the true value.

15.4 Two method blanks shall be prepared ensuring that an adequate method blank volume is present for a minimum of

seven repetitive analysis. The standard deviation of the method blank is used to determine the minimum detectable concentration of each sample and control in the batch.

15.5 A Laboratory Control Sample should be analyzed with each batch of samples at a minimum frequency of 10 %.

15.6 If the QC for the sample batch is not within the established control limits, reanalyze the samples or qualify the results with the appropriate flags, or both (D 5847)

15.7 Blind control samples should be submitted by an outside agency in order to determine the laboratory performance capabilities.

16. Keywords

16.1 elements; inductively-coupled argon plasma atomic emission spectroscopy; simultaneous determination

TABLE 8 Regression Equations for Bias and Precision, µg/L, Reagent Water versus Surface Water (Manganese, Molybdenum, Nickel, Selenium)

 NOTE 1— X = mean recovery; C = true value for the concentration.

Water Type	Manganese	Molybdenum	Nickel	Selenium
Total Digestion				
Applicable concentration range	(17 to 943)	(73 to 1094)	(43 to 943)	(83 to 755)
Reagent water, hard				
Single-analyst precision	$S_o = 0.02X + 0.50$	$S_o = 0.04X + 0.97$	$S_o = 0.00X + 9.15$	$S_o = 0.04X + 3.82$
Overall precision	$S_t = 0.04X + 0.93$	$S_t = 0.08X - 1.77$	$S_t = 0.04X + 6.46$	$S_t = 0.11X + 13.14$
Bias	$X = 0.97C - 1.46$	$X = 0.97C - 2.93$	$X = 0.98C - 2.93$	$X = 0.92C - 0.48$
Surface water, hard				
Single-analyst precision	$S_o = 0.01X + 3.44$	$S_o = 0.06X - 2.60$	$S_o = 0.01X + 3.39$	$S_o = 0.03X + 7.53$
Overall precision	$S_t = 0.03X + 4.69$	$S_t = 0.09X - 2.27$	$S_t = 0.03X + 6.43$	$S_t = 0.13X + 15.91$
Bias	$X = 0.95C + 2.06$	$X = 0.96C + 1.30$	$X = 0.98C + 1.17$	$X = 0.91C + 6.31$
Total-Recoverable Digestion				
Applicable concentration range	(17 to 943)	(73 to 1094)	(43 to 943)	(83 to 755)
Reagent water, soft				
Single-analyst precision	$S_o = 0.04X + 0.29$	$S_o = 0.06X + 0.58$	$S_o = 0.05X + 1.98$	$S_o = 0.06X + 4.00$
Overall precision	$S_t = 0.06X + 0.86$	$S_t = 0.06X + 6.49$	$S_t = 0.06X + 3.33$	$S_t = 0.14X + 15.64$
Bias	$X = 0.98C - 0.78$	$X = 0.99C - 6.78$	$X = 1.00C - 0.66$	$X = 0.97C + 0.36$
Reagent water, soft				
Single-analyst precision	$S_o = 0.04X + 2.90$	$S_o = 0.02X + 4.55$	$S_o = 0.04X + 0.35$	$S_o = 0.05X + 3.05$
Overall precision	$S_t = 0.07X + 5.85$	$S_t = 0.02X + 7.08$	$S_t = 0.05X + 3.29$	$S_t = 0.12X - 0.02$
Bias	$X = 0.97C - 0.02$	$X = 1.02C - 5.90$	$X = 0.96C + 4.20$	$X = 0.95C - 3.25$

TABLE 9 Regression Equations for Bias and Precision, µg/L, Reagent Water versus Surface Water (Silver, Thallium, Vanadium, Zinc)

 NOTE 1— X = mean recovery; C = true value for the concentration.

Water Type	Silver	Thallium	Vanadium	Zinc
Total Digestion				
Applicable concentration range	(17 to 189)	(126 to 953)	(41 to 1877)	(68 to 759)
Reagent water, hard				
Single-analyst precision	$S_o = 0.22X - 2.05$	$S_o = 0.00X + 24.72$	$S_o = 0.03X - 0.28$	$S_o = 0.00X + 8.29$
Overall precision	$S_t = 0.64X - 6.71$	$S_t = 0.07X + 25.10$	$S_t = 0.05X + 3.80$	$S_t = 0.02X + 10.91$
Bias	$X = 0.29C + 9.78$	$X = 0.93C - 16.28$	$X = 0.97C - 1.85$	$X = 0.97C - 3.04$
Surface water, hard				
Single-analyst precision	$S_o = 0.16X - 0.33$	$S_o = 0.06X - 1.59$	$S_o = 0.02X + 4.71$	$S_o = -0.00X + 5.17$
Overall precision	$S_t = 0.46X - 3.07$	$S_t = 0.06X + 3.70$	$S_t = 0.06X + 3.10$	$S_t = 0.05X + 7.17$
Bias	$X = 1.02C - 4.12$	$X = 0.90C - 15.59$	$X = 1.00C - 2.07$	$X = 0.98C + 0.57$
Total-Recoverable Digestion				
Applicable concentration range	(17 to 189)	(126 to 953)	(41 to 1877)	(68 to 759)
Reagent water, soft				
Single-analyst precision	$S_o = 0.15X + 1.35$	$S_o = 0.02X + 33.81$	$S_o = 0.05X + 0.78$	$S_o = 0.06X + 2.52$
Overall precision	$S_t = 0.83X - 12.00$	$S_t = 0.07X + 30.95$	$S_t = 0.06X + 5.41$	$S_t = 0.05X + 7.98$
Bias	$X = 0.23C + 13.92$	$X = 0.87C + 12.93$	$X = 0.97C - 1.32$	$X = 1.02C - 8.32$
Reagent water, soft				
Single-analyst precision	$S_o = 0.07X + 0.17$	$S_o = 0.14X - 1.80$	$S_o = 0.01X + 1.86$	$S_o = 0.01X + 9.04$
Overall precision	$S_t = 0.08X + 1.45$	$S_t = 0.15X - 0.58$	$S_t = 0.05X + 4.97$	$S_t = 0.00X + 16.57$
Bias	$X = 0.79C + 3.44$	$X = 0.84C - 6.86$	$X = 0.98C - 1.14$	$X = 1.01C - 8.67$

APPENDIXES

(Nonmandatory Information)

X1. ADDITIONAL TEST ELEMENTS BY INDUCTIVELY-COUPLED ARGON PLASMA ATOMIC EMISSION SPECTROSCOPY

X1.1 Table X1.1 is provided as a guide for suggested wavelengths and detection limits.

TABLE X1.1 Suggested Wavelengths and Estimated Detection Limits⁵

Element	Wavelength, nm ^A	Estimated detection limit, µg/L ^B
Barium	455.403	2
Calcium	317.933	10
Lithium	670.784	4
Potassium	766.491	C
Silica	288.158	27
Sodium	588.995	29

^A The wavelengths listed are recommended because of their sensitivity and overall acceptance. Other wavelengths may be substituted if they can provide the needed sensitivity and are treated with the same corrective techniques for spectral interference (see 6.1.1).

^B The estimated detection limits as shown are taken from Winge, Fassel, et al. They are given as a guide for approximate detection limits. The actual method instrumental detection limits are sample dependent and may vary as the sample matrix varies (see 3.1.4).

^C Highly dependent on operating conditions and plasma position.

X1.2 Table X1.2 is provided as a guide for preparation of metal stock solutions.

TABLE X1.2 Preparation of Metal Stock Solution^{A,B}

Element (Compound)	Weight, g	Solvent
BaCl ₂ ^C	0.1516	HCl (1 + 1)
CaCO ₃ ^D	0.2498	Water + HCl (1 + 1)
Li ₂ CO ₃	0.1907	HNO ₃ (1 + 1)
KCl	0.5323	Water
Na ₂ SiO ₃ ·5H ₂ O	0.3531	Water
NaCl	0.2542	Water

^A Metal stock solutions, 1.00 mL = 100 µg of metal. Dissolve the listed weights of each compound or metal in 20 mL of specified solvent and dilute to 1 L. The metals may require heat to increase rate of dissolution.

^B Where water is used as the solvent, acidify with 10 mL of HNO₃(sp gr 1.42) and dilute to 1 L. See Section 8 for concentration of acids. Commercially available standards may be used. Alternate salts or oxides may also be used.

^C Dry for 1 h at 180°C.

^D Dry for 1 h at 180°C. Add to approximately 600 mL of water and dissolve cautiously with a minimum of dilute HCl. Dilute to 1 L with water.

X2. PRECISION AND BIAS

X2.1 Study data sets for potassium, lithium, sodium, thallium, and silicon were limited due to either the small number of laboratories reporting data for the element or to an unusually high percentage of rejected data. Regression equations and summary statistics for these elements must, therefore, be used with prudence.

X2.2 Low concentration level data for aluminum, boron, and silicon were affected by contamination of the spiking material from the borosilicate glass ampules used in the study. Precision and bias for low concentration spikes for these elements were poorer than expected due to this difficulty.

X2.3 High levels of some elements in specific effluents made evaluation of data for precision and bias difficult. This

problem was inherent in the study design and selection of real world effluents.

X2.4 The following elements have shown some matrix effect of practical importance due to water type: aluminum, barium, beryllium, boron, cobalt, copper, iron, magnesium, manganese, nickel, selenium, silver, strontium, vanadium, and zinc.

X2.5 Digestion was shown to have an effect on accuracy or precision or both on some of the elements studied.

X2.6 High solids or MAK-type nebulization for high dissolved solids samples was less prone to difficulties than standard, fixed cross-flow or concentric nebulizers.

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<231> HEAVY METALS

This test is provided to demonstrate that the content of metallic impurities that are colored by sulfide ion, under the specified test conditions, does not exceed the *Heavy metals* limit specified in the individual monograph in terms of the percentage (by weight) of lead in the test substance, as determined by concomitant visual comparison (see *Visual Comparison* in the section *Procedure* under *Spectrophotometry and Light-Scattering* < 851 >) with a control prepared from a *Standard Lead Solution*. [NOTE — Substances that typically will respond to this test are lead, mercury, bismuth, arsenic, antimony, tin, cadmium, silver, copper, and molybdenum.]

Determine the amount of heavy metals by *Method I*, unless otherwise specified in the individual monograph. *Method I* is used for substances that yield clear, colorless preparations under the specified test conditions. *Method II* is used for substances that do not yield clear, colorless preparations under the test conditions specified for *Method I*, or for substances that, by virtue of their complex nature, interfere with the precipitation of metals by sulfide ion, or for fixed and volatile oils. *Method III*, a wet-digestion method, is used only in those cases where neither *Method I* nor *Method II* can be utilized.

Special Reagents

Lead Nitrate Stock Solution — Dissolve 159.8 mg of lead nitrate in 100 mL of water to which has been added 1 mL of nitric acid, then dilute with water to 1000 mL. Prepare and store this solution in glass containers free from soluble lead salts.

Standard Lead Solution — On the day of use, dilute 10.0 mL of *Lead Nitrate Stock Solution* with water to 100.0 mL. Each mL of *Standard Lead Solution* contains the equivalent of 10 µg of lead. A comparison solution prepared on the basis of 100 µL of *Standard Lead Solution* per g of substance being tested contains the equivalent of 1 part of lead per million parts of substance being tested.

Method I

pH 3.5 Acetate Buffer — Dissolve 25.0 g of ammonium acetate in 25 mL of water, and add 38.0 mL of 6 N hydrochloric acid. Adjust, if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 mL, and mix.

Standard Preparation — Into a 50-mL color-comparison tube pipet 2 mL of *Standard Lead Solution* (20 µg of Pb), and dilute with water to 25 mL. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, dilute with water to 40 mL, and mix.

Test Preparation — Into a 50-mL color-comparison tube place 25 mL of the solution prepared for the test as directed in the individual monograph; or, using the designated volume of acid where specified in the individual monograph, dissolve and dilute with water to 25 mL the quantity, in g, of the substance to be tested, as calculated by the formula:

$$2.0 / (1000L),$$

in which *L* is the *Heavy metals* limit, in percentage. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, dilute with water to 40 mL, and mix.

Monitor Preparation — Into a third 50-mL color-comparison tube place 25 mL of a solution prepared as directed for *Test Preparation*, and add 2.0 mL of *Standard Lead Solution*. Adjust with 1 N acetic acid or 6 N ammonium hydroxide to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, dilute with water to 40 mL, and mix.

Procedure — To each of the three tubes containing the *Standard Preparation*, the *Test Preparation*, and the *Monitor Preparation*, add 2 mL of *pH 3.5 Acetate Buffer*, then add 1.2 mL of thioacetamide –glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*, and the intensity of the color of the *Monitor Preparation* is equal to or greater than that of the *Standard Preparation*. [NOTE — If the color of the *Monitor Preparation* is lighter than that of the *Standard Preparation*, use *Method II* instead of *Method I* for the substance being tested.]

Method II

pH 3.5 Acetate Buffer — Prepare as directed under *Method I*.

Standard Preparation — Prepare as directed under *Method I*.

Test Preparation — Use a quantity, in g, of the substance to be tested as calculated by the formula:

$$2.0 / (1000L),$$

in which *L* is the *Heavy metals* limit, in percentage. Transfer the weighed quantity of the substance to a suitable crucible, add sufficient sulfuric acid to wet the substance, and carefully ignite at a low temperature until thoroughly charred. (The crucible may be loosely covered with a suitable lid during the charring.) Add to the carbonized mass 2 mL of nitric acid and 5 drops of sulfuric acid, and heat cautiously until white fumes no longer are evolved. Ignite, preferably in a muffle furnace, at 500° to 600°, until the carbon is completely burned off. Cool, add 4 mL of 6 N hydrochloric acid, cover, digest on a steam bath for 15 minutes, uncover, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochloric acid, add 10 mL of hot water, and digest for 2 minutes. Add 6 N ammonium hydroxide dropwise, until the solution is just alkaline to litmus paper, dilute with water to 25 mL, and adjust with 1 N acetic acid to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator. Filter if necessary, rinse the crucible and the filter with 10 mL of water, combine the filtrate and rinsing in a 50-mL color-comparison tube, dilute with water to 40 mL, and mix.

Procedure — To each of the tubes containing the *Standard Preparation* and the *Test Preparation*, add 2 mL of *pH 3.5 Acetate Buffer*, then add 1.2 mL of thioacetamide–glycerin base TS, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the solution from the *Test Preparation* is not darker than that of the solution from the *Standard Preparation*.

Method III

pH 3.5 Acetate Buffer — Prepare as directed under *Method I*.

Standard Preparation — Transfer a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to a clean, dry, 100-mL Kjeldahl flask, and add a further volume of nitric acid equal to the incremental volume of nitric acid added to the *Test Preparation*. Heat the solution to the production of dense, white fumes, cool, cautiously add 10 mL of water and, if hydrogen peroxide was used in treating the *Test Preparation*, add a volume of 30 percent hydrogen peroxide equal to that used for the substance being tested, and boil gently to the production of dense, white fumes. Again cool,

cautiously add 5 mL of water, mix, and boil gently to the production of dense, white fumes and to a volume of 2 to 3 mL. Cool, dilute cautiously with a few mL of water, add 2.0 mL of *Standard Lead Solution* (20 µg of Pb), and mix. Transfer to a 50-mL color-comparison tube, rinse the flask with water, adding the rinsing to the tube until the volume is 25 mL, and mix.

Test Preparation —

If the substance is a solid — Transfer the quantity of the test substance specified in the individual monograph to a clean, dry, 100-mL Kjeldahl flask. [NOTE — A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and add a sufficient quantity of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid to moisten the substance thoroughly. Warm gently until the reaction commences, allow the reaction to subside, and add additional portions of the same acid mixture, heating after each addition, until a total of 18 mL of the acid mixture has been added. Increase the amount of heat, and boil gently until the solution darkens. Cool, add 2 mL of nitric acid, and heat again until the solution darkens. Continue the heating, followed by addition of nitric acid until no further darkening occurs, then heat strongly to the production of dense, white fumes. Cool, cautiously add 5 mL of water, boil gently to the production of dense, white fumes, and continue heating until the volume is reduced to a few mL. Cool, cautiously add 5 mL of water, and examine the color of the solution. If the color is yellow, cautiously add 1 mL of 30 percent hydrogen peroxide, and again evaporate to the production of dense, white fumes and a volume of 2 to 3 mL. If the solution is still yellow in color, repeat the addition of 5 mL of water and the peroxide treatment. Cool, dilute cautiously with a few mL of water, and rinse into a 50-mL color-comparison tube, taking care that the combined volume does not exceed 25 mL.

If the substance is a liquid — Transfer the quantity of the test substance specified in the individual monograph to a clean, dry, 100-mL Kjeldahl flask. [NOTE — A 300-mL flask may be used if the reaction foams excessively.] Clamp the flask at an angle of 45°, and cautiously add a few mL of a mixture of 8 mL of sulfuric acid and 10 mL of nitric acid. Warm gently until the reaction commences, allow the reaction to subside, and proceed as directed under *If the substance is a solid*, beginning with “add additional portions of the same acid mixture.”

Procedure — Treat the *Test Preparation* and the *Standard Preparation* as follows: Adjust the solution to a pH between 3.0 and 4.0, using short-range pH indicator paper as external indicator, with ammonium hydroxide (a dilute ammonia solution may be used, if desired, as the specified range is approached), dilute with water to 40 mL, and mix.

To each tube add 2 mL of *pH 3.5 Acetate Buffer*, then add 1.2 mL of *thioacetamide-glycerin base TS*, dilute with water to 50 mL, mix, allow to stand for 2 minutes, and view downward over a white surface: the color of the *Test Preparation* is not darker than that of the *Standard Preparation*.

* In those countries or jurisdictions where thioacetamide cannot be used, add 10 mL of freshly prepared *hydrogen sulfide TS* to each of the tubes, mix, allow to stand for 5 minutes, and view downward over a white surface

Stepan Company Analytical Method

Stepan Company
Northfield, Illinois 60093
(847) 446-7500

SM 538-A
Total Pages: 6

Low Level Methanol and Ester in Glycerides by Headspace Gas Chromatography

Research Method	Accepted: 05/14/2003	Supercedes:
Author: A. Koberda	Aprv'd: (Anl) <i>David M. Koberda</i> (QA)	<i>David E. Quinn</i>

SCOPE: This automated headspace gas chromatographic (HSGC) method determines methanol and C25 methyl esters as methyl caprylate (C8) in glycerides made from methyl esters such as Stepan GTC up to 15 ppm. The results are expressed in ppm (ug/g). The estimated detection limits is 0.2 ppm for methanol and 2.5 ppm ester (see Remark 1).

SUMMARY: A GTC (made from methyl esters) sample is analyzed against external calibration using n-propanol as an internal standard. The external standards are prepared using samples made from fatty acids such as NEOBEE® M-5 or NEOBEE® 1053 (surrogate matrix). The standards/surrogate matrix is weighed into separate headspace vials (3) followed by an addition of internal standard solution and known amounts of methanol and C25 ester (spiking). The sample solution is prepared with internal standard only. The sample and standards solutions are analyzed by headspace gas chromatography on a capillary column using a flame ionization detector. The methanol and C25 content are determined from 3-level calibration curve.

SAFETY: METHANOL, n-PROPANOL, and METHYL ESTERS are FLAMMABLE and POISON. Avoid eye and skin contact. Avoid open flames and sparks. Wear proper personal protective equipment. Work in a hood or well ventilated area.

This method may include the use of potentially hazardous materials. Refer to the MSDS for additional handling and safety information.

Follow appropriate federal, state, and local regulations for proper waste disposal.

APPARATUS:

1. Gas Chromatograph, Agilent 6890 GC or equivalent equipped with an FID, a volatile interface (as an inlet) and electronic pressure control (EPC) and a computer workstation
2. Programs on computer workstation, HP GC software, version A.03.03 or higher; Microsoft Windows, version 4.0 or higher; Microsoft Excel, version 5.0 or higher
3. Headspace analyzer, HP 7694 or HP 19395 or equivalent, with a 1 mL sample loop
4. Column, DB-WAXETR, 30 m x 0.25 mm, df = 0.25 microns
5. Flask, volumetric, 10 mL
6. Pipet, positive displacement, 10 uL, 25 uL, 250 uL and 5 mL
7. Analytical balance, +/- 0.0001g
8. Headspace vials, 20 mL, Teflon/butyl septa, crimp caps, open top, vial crimper and decrimper
9. Ultrasonic bath or mechanic shaker (Vortex), optional
10. Dark glass containers, suitable size

REAGENTS: 1. Methanol, HPLC grade

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2. C25 methyl esters of known composition, C8 content 53-62% by area.
3. 1-Propanol (n-propanol), purity > 99%
4. NEOBEE® 1053 or NEOBEE® M-5 sample, methanol and ester free material "GTC solvent"

PROCEDURE:

A. INSTRUMENT CONDITIONS for COMPUTER CONTROLLED 6890 GC (with EPC):

1. Install the capillary DB-WAXETR into the inlet and detector of the GC oven according to the column and instrument manufacturer's recommendations.
2. Program the 6890 GC using the following pages of Instrument Edit screen as follows:

Select Injection Source: Manual and enter the location of transfer line (e.g. Back).

Edit Parameters:

Columns (Carrier Flows): Install electronically an appropriate column (e.g. Back or "2")

a. Mode: Ramp Flow

Select the inlet and detector in use.

b. Inlet (Injection Port, Volatile Interface):

Temperature	220 °C
Split Ratio	30:1

c. Column (Carrier Flows):

Mode: Ramp Flow

Flow 1	1 mL/minute 11.6 psi @ 40 °C
Time 1 (Hold)	0.05 minutes
Ramp 1	5.0 mL/minute/minute
Flow 2	3 mL/minute
Time 2 (Hold)	0.1 minutes
Ramp 2	10.0 mL/minute/minute
Flow 3	1 mL/minute
Time 3 (Hold)	15.0 minutes
Ramp 3	10.0 mL/minute/minute
Flow 4	3 mL/minute
Time 4 (Hold)	5 minutes

d. Oven:

Initial Temperature	40 °C
Hold Time	6 minute
Ramp 1	10 °C/minute
Temperature 2	140 °C
Time 2	0.0 minutes
Ramp 2	60 °C/minute
Temperature 3	240 °C
Time 3	2.0 minutes
Run Time	19.7 minutes

e. Signal:

Select Save Data for the FID in use
Select Rate: 10 Hz

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f. FID Detector:

Temperature 250 °C
Air Flow 350 mL/minute
Hydrogen Flow 30 mL/minute
Nitrogen Flow (make-up gas) 20 mL/minute

g. Aux:

Select Aux Channel designated for vial pressure
Initial 15 psi, Hold 3.0 minutes
Ramp 1 15 psi to 5 psi, Hold 10.0 minutes

Options:

Select psi units pressure for Aux Channel

3. Program the HP 7694 headspace sampler conditions as follows:

Sample Oven 120 °C
Sample Valve 140 °C
Transfer Line 150 °C

Timing (sampling) in minutes for headspace autosampler:

GC Cycle 26.0
Sample Equilibration 10.0
Vial Pressurization 0.20
Loop Fill 0.20
Loop Equilibration 0.05
Sample Inject 1
Oven Stabilization 1.0
Agitation High
Injections per Vial 1

B. STANDARD PREPARATION:

Stock Solution of Methanol and C25 Esters (about 1% or 10 ug/uL for each component):

1. Accurately weigh into a 10 mL volumetric flask, 0.08-0.12g, +/- 0.0001g, of methanol and 0.12-0.15g, +/- 0.0001g, of C25 methyl ester. Dilute to volume with GTC solvent and mix thoroughly. This is the Standard Stock Solution (SSS). Store the solution in a refrigerator up to 4 weeks.

Working Solution of Methanol and C25 Esters:

1. Add 250 uL (by positive displacement pipet) of the SSS to the 10 mL volumetric flask. Dilute to volume with GTC solvent and mix thoroughly. This is the Working Solution of methanol and C25 ester (WS). Store the solution in a refrigerator up to 4 weeks.

Internal Standard Solution (ISTD):

1. In a suitable vial mix together 5 uL of n-propanol and 5 mL of GTC solvent. Both components are added by positive displacement pipet. This is the internal standard solution.

C. CALIBRATION STANDARD PREPARATION:

1. Accurately weigh 2.0g, +/- 0.004g, of GTC solvent (methanol and ester free material) into 3 separate headspace vials.
2. Add 10 uL of the ISTD solution to each vial. Dissolve the samples using Vortex mixer or an ultrasonic bath.
3. Add methanol/C25 working solution in 25 uL increments using a positive displacement micropipet:

Level 1	25 uL (MeOH: 3 ppm, C25: 6 ppm)
Level 2	50 uL (MeOH: 6 ppm, C25: 12 ppm)
Level 3	75 uL (MeOH: 9 ppm, C25: 18 ppm)

4. Close the vial and shake vigorously (or use Vortex mixer) for 10 seconds. These are the methanol/C25 calibration solutions.

D. SAMPLE PREPARATION:

1. Accurately weigh 2.0g, +/- 0.4mg, of the sample for methanol analysis into a headspace vial.
2. Add 10 uL of the ISTD solution to the vial. Close the vial. Mix the sample solution using Vortex mixer an ultrasonic bath. This is the sample solution.

E. STANDARD and SAMPLE ANALYSIS:

For routine analysis at QC lab, omit Steps 1-8.

1. Program the GC as stated in PROCEDURE A, INSTRUMENT CONDITIONS, using GC manual or on-line help. Select the ISTD Report from Data Analysis. Save the procedure as a Method under the specific name.
2. Inject the following Methanol and C25 Calibration Solutions (3 vials)

Level 1	25 uL
Level 2	50 uL
Level 3	75 uL
3. Calculate the amount of methanol and C25 added, in ppm, to each calibration solution (Level 1 through 3) as stated in the CALCULATIONS section.
4. Using Data Analysis/Graphics features identify the methanol and n-propanol and C8 ester peaks according to the attached GC profile of Appendices 1.
5. Set up the integration events so only these three peaks (MeOH, C8 and n-PrOH) are integrated.
6. Construct a 3-level calibration table for MeOH and C25 with n-PrOH as an internal standard. Enter methanol and C25 amounts for Level 1, 2 and 3 calculated in ppm. Enter the amount "1" for n-PrOH, ISTD. Make sure the integration was done correctly for both components at each level. Select the ISTD Report and Printer as a Report destination from Data Analysis. Save the procedure.
8. Select Data Acquisition and Data Analysis from the method's Run Time

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Checklist.

9. Inject the sample solution (PROCEDURE D, Step 2) according to Run Time Checklist of the method. The quantitative results, as ISTD, will be printed automatically upon completion of the run.

CALCULATIONS:

Using Methanol as an example performed calculations in the same manner for C25 methyl ester.

1. Calculate the Methanol (MeOH) concentration in the stock solution (added to 10 mL vol. flask):

$$\text{MeOH in SSS} = \text{WT/MeOH} \times \text{P/MeOH} \times 100$$

Where:

MeOH in SSS = MeOH Concentration in mg/mL or ug/uL in Stock Solution

WT/MeOH = Weight, g of MeOH in Stock Solution

P/MeOH = Purity of MeOH Reagent, Expressed as a Decimal Fraction

100 = Dilution/Unit Conversion Factor

2. Calculate the Methanol (MeOH) concentration in the working solution, WS, (250 uL of SSS added to 10 mL vol. flask):

$$\text{MeOH in WS} = \text{MeOH in SSS} \times (0.250 / 10)$$

Where:

MeOH in SSS = MeOH Concentration in mg/mL or ug/uL in Stock Solution

MeOH in WS = MeOH Concentration in mg/mL or ug/uL in Stock Solution

(0.250 / 10) = Dilution Factor

3. Calculate the amount of MeOH added to the calibration standards (added via spikes) in %:

$$\text{MeOH/Level 1} = \text{MeOH in WS} \times 25/2$$

$$\text{MeOH/Level 2} = \text{MeOH in WS} \times 50/2$$

$$\text{MeOH/Level 3} = \text{MeOH in WS} \times 75/2$$

Where:

MeOH in WS = MeOH Concentration in Working Solution (ug/uL)

25, 50 and 75 = Amount of Spiking Solution Added to Each Calibration Vial in uL

2 = Sample Weight, g

4. The amount of MeOH in a sample is read from the curve using area ratio (MeOH/n-PrOH of the sample run).

PRECISION and ACCURACY:**PRECISION:**

The precision of the procedure, measured as a relative standard deviation was found to be less than 10% for both components for 3 ppm methanol and 6 ppm C25 levels.

ACCURACY:

Agent #2593-58 (undeodorized Stepan GTC) was spiked with known amount of MeOH and C25 and analyzed in 5 replicates; Analyzed, ppm are averages of 5 determinations.

Added, ppm Initial, ppm Init. + Added, ppm Analyzed, ppm Recovered, %

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MeOH	2.46	0.37	2.83	3.11	109.8
C25	4.92	1.43	6.35	6.14	96.8

The initial amount (before spiking) of both components was roughly estimated from linear regression using standard addition technique. Methanol was detected, while C8 ester was below LOD for this sample.

REMARKS:

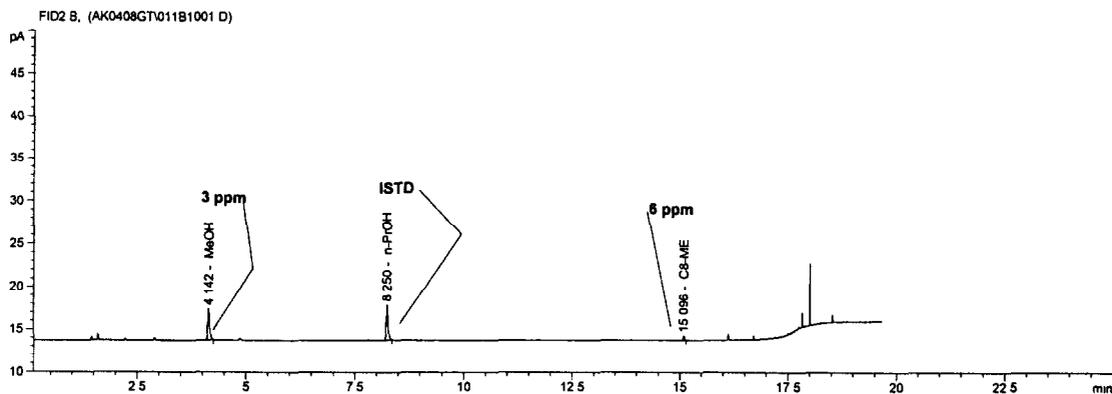
1. The LOD and LOQ were determined from the signal/noise (S/N) ratio and found to be 0.5 and 0.7 ppm for methanol, while for C25 ester (as C8), 2.5 and 5.0 ppm respectively. The C8 ester concentration was 53.7% by weight.

REFERENCES:

1. A. Koberda, Stepan Laboratory Notebook #0048, pp. 18-20, 25,27, 30-34.
2. Koberda, A., "Methanol Methyl Esters in NEOBEE® M-5 ", Stepan Library Document #02130, April 03, 2002.
3. Koberda, A., "Stepan Maywood: Stepan GTC, C25 and Methanol Analysis", Stepan Library Document #03176, April 28, 2003.

APPENDICES:

Appendix 1 = Spiked Undeodorized Sample of Stepan GTC; HS-GC/FID Profile

Graphics:

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Stepan



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Specification Name: PD015 MANUFACTURING STANDARD

Manufacturing Specification

Issue: 1 (18-Feb-2000)

Customer Specification

Approvals: Jeff Romano

Raw Materials Specification

Tradenames: STEPAN C-25

Material Name: STEPAN C-25

Mid4: 0725

Material Type: METHYL ESTER

Lot Plans:

Lot Plan & Use:	PD015 ML	MANUFACTURING STAN
	PD015 MW	MANUFACTURING STAN
	PD015 WN	MANUFACTURING STAN

Sample Plans:

Plants

Study:	FINISHED GOODS	ML	MW	WN
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Description	Limits	Measure Unit
110-0 APPEARANCE @ 100F	Clear, Water White Liquid	
022-0 WATER (%)	0.1 Max.	%
050-0 ACID VALUE	1.5 Max.	MG KOH/G
518-0* IODINE VALUE	1.0 Max.	COMPLIES
006-B COLOR APHA	50 Max.	
505-0 HEAT STABILITY APHA	75 Max.	
114-B FLASH POINT (F)	100-200 F (SETA)	F
103-F CARBON DISTRIBUTION (S/B)	%	
103-F C06	5 Max.	%
103-F C08	53-62	%
103-F C10	33-41	%
103-F C12 & ABOVE	2 Max.	%
167-C NIR ID	Passes	

Informative Data

-- REVISION INFORMATION
P-COM# - PNF-00046
REASON FOR REVISION - CHANGE IODINE VALUE TO COMPLIES

-- COMPOSITION TEXT
METHYL ESTER MIXTURE:
C6 5% MAX.



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Specification Name: RG108 MANUFACTURING STANDARD

Manufacturing Specification

Issue: 3 (07-Oct-2002)

Customer Specification

Approvals: John Schulze

Raw Materials Specification

Tradenames: GLYCERINE USP KOSHER

Material Name: GLYCERINE USP KOSHER

Mid4: 5236

Material Type: ESTERS/OILS

Lot Plans:

Lot Plan & Use: RG108 ML MANUFACTURING STAN
 RG108 MW MANUFACTURING STAN

Sample Plans:

Study: RAW MATERIALS ML MW

Plants

Description	Limits	Measure Unit
006-A COLOR APHA	10 MAX.	
545-0 ACIDITY	0.01 MAX. (AS ACETIC)	%
506-0 SPECIFIC GRAVITY	1.2600/1 2640 (@25C)	
509-0* SULFURIC ACID APHA	75 Max. (5%)	COMPLIES
110-0 APPEARANCE @ 25C	CLEAR LIQUID	
501-0 WATER	0.5% MAX.	%
000-0 KOSHER GRADE	PASSES	
000-0 FREE GLYCERINE	99 5 MIN.	%
167-C NIR ID	PASSES	

Informative Data

-- Revision Information
 P-Com: PMW 02614
 Reason for Revision: Change Sulfuric Acid APHA to Compliance.

-- COMPOSITION TEXT
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 2 2

-- INFORMATIVE DATA
 ARSENIC 1.5 PPM
 CHLORINATED COMPOUNDS 30 PPM
 CHLORINE 10 PPM
 HEAVY METALS 5 PPM



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Specification Name: RK102 MANUFACTURING STANDARD Manufacturing Specification
Issue: 1 (16-Mar-2000) Customer Specification
Approvals: Don Verbic Raw Materials Specification

Material Name: FASCAT 4100 **Mid4:** 5181
Material Type: CATALYSTS/STABILIZERS

Lot Plans:

Lot Plan & Use: RK102 ML MANUFACTURING STAN
 RK102 MW MANUFACTURING STAN

Sample Plans:

Study: RAW MATERIALS ML MW

Plants

Description	Limits	Measure Unit
000-0 TIN (%)	54.0-59.6	%
110-0 APPEARANCE @ 25C	WHITE POWDER	
021-0 SOLIDS	3.0 Max.(As Loss On Drying)	%

Informative Data

-- COMPOSITION TEXT -----
 HYDRATED MONOBUTYLTIN OXIDE.