AOAC RESEARCH INSTITUTE

AOAC Official Methods of Analysis℠ (OMA)

AOAC EXPERT REVIEW PANEL

FOR FERTILIZERS

AOAC MIDYEAR MEETING

WEDNESDAY, MARCH 15, 2017
1:00PM – 4:00PM

Gaithersburg Marriott Washingtonian Center
9751 Washingtonian Boulevard
Gaithersburg, MD 20878 USA

AOAC *OFFICIAL METHODS OF ANALYSIS*^{SM}

The *Official Methods of Analysis*^{SM} (OMA) program is AOAC INTERNATIONAL's premier methods program. The program evaluates chemistry, microbiology, and molecular biology methods. It also evaluates traditional benchtop methods, instrumental methods, and proprietary, commercial, and/or alternative methods. In 2011, AOAC augmented the *Official Methods*^{SM} program by including an approach to First Action *Official Methods*^{SM} status that relies on gathering the experts to develop voluntary consensus standards, followed by collective expert judgment of methods using the adopted standards.

The OMA program has undergone a series of transitions in support of AOAC's collaborations, evolving technology, and evolving technical requirements. Methods approved in this program have undergone rigorous scientific and systematic scrutiny such that analytical results by methods in the *Official Methods of Analysis of AOAC INTERNATIONAL* are deemed to be highly credible and defensible.

On September 7, 2012, AOAC INTERNATIONAL further clarified the AOAC *Official Methods*^{SM} program by transitioning the conformity assessment component of the *Official Methods*^{SM} program into the AOAC Research Institute. The AOAC Research Institute now administers the AOAC *Official Methods*^{SM} program for all proprietary, single and sole source methods. Methods submitted through the PTM-OMA harmonized process also will be reviewed through the AOAC Research Institute. All methods in the AOAC *Official Methods*^{SM} program are now reviewed by Expert Review Panels for First Action AOAC *Official Methods of Analysis*^{SM} status.

EXPERT REVIEW PANEL (ERP)

The AOAC Expert Review Panels (ERPs) are a key part of AOAC INTERNATIONAL's Method Approval Process. AOAC ERPs are authorized to adopt candidate methods as First Action *Official Methods* and to recommend adoption of these methods to Final Action *Official Methods* status. Scientists are recruited to serve on ERPs in a variety of ways. Normally, a call for experts is published at the same time as a call for methods is posted. Interested scientists are invited to submit their *curriculum vitae* (CV) for consideration. Advisory panel, stakeholder panel, and working group members may make recommendations to AOAC for ERP members. All CVs are reviewed and evaluated for expertise by the AOAC Chief Scientific Officer (CSO) and then to the AOAC Official Methods Board for formal review. The composition of the ERP must be fulfilled with qualified subject matter experts representing various perspectives. Please refer to our Call for Experts on the AOAC homepage for further information.

AOAC INTERNATIONAL
2275 Research Blvd, Suite 300
Rockville, Maryland 20850
Phone: (301) 924-7077

AOAC RESEARCH INSTITUTE

AOAC *Official Methods of Analysis*℠ (OMA) Expert Review Panel for Fertilizers

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EXPERT REVIEW PANEL (ERP) FOR FERTILIZERS

Gaithersburg Marriott Washingtonian Center
9751 Washingtonian Boulevard, Gaithersburg, MD 20878 USA

WEDNESDAY, MARCH 15, 2017
1:00PM – 4:00PM

MEETING AGENDA

Expert Review Panel Chair: Dr. William Hall, Mosiac

I. Welcome and Introductions
Expert Review Panel Co-Chairs

II. Review of AOAC Volunteer Policies & Expert Review Panel Process Overview and Guidelines
Deborah McKenzie, Senior Director, Standards Development and Method Approval Processes, AOAC INTERNATIONAL and AOAC Research Institute

III. Review of Methods
For each method the assigned ERP members will present a review of the proposed collaborative study manuscript, after which the ERP will discuss the method and render a decision on the status for each method.

1) **OMAMAN-28: Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganeses, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry Detection: Single Laboratory Validation**
Study Director: Sharon Webb, University of Kentucky, Division of Regulatory Services, 103 Regulatory Services Bldg, Lexington , Kentucky 40546-0275

2) **AOAC OFFICIAL METHOD 959.03: UREA IN FERTILIZERS [FINAL ACTION 1960]**
Study Director: Michael Hojjatie, Ph.D., 2248 W. Lower Buckeye, Phoenix, AZ 85009

IV. Discuss Final Action Requirements for First Action Official Methods (if applicable)
ERP will discuss, review and track First Action methods for 2 years after adoption, review any additional information (i.e., additional collaborative study data, proficiency testing, and other feedback) and make recommendations to the Official Methods Board regarding Final Action status.

V. Follow –Up of Previously Reviewed Methods

A. **OMAMAN-24: Determination of Total Sulfur in Fertilizers by High Temperature Combustion**
Co-Study Directors: Tyson Rowland and Jean Bernius, elementar Americas, 520 Fellowship Road, Suite D-408, Mt. Laurel, New Jersey 08054

VI. Next Steps and Upcoming Meetings

VII. Adjournment

**Agenda is subject to change. V1

AOAC INTERNATIONAL

POLICY AND PROCEDURES ON

VOLUNTEER CONFLICT OF INTEREST

Statement of Policy

While it is not the intention of AOAC INTERNATIONAL (AOAC) to restrict the personal, professional, or proprietary activities of AOAC members nor to preclude or restrict participation in Association affairs solely by reason of such activities, it is the sense of AOAC that conflicts of interest or even the appearance of conflicts of interest on the part of AOAC volunteers should be avoided. Where this is not possible or practical under the circumstances, there shall be written disclosure by the volunteers of actual or potential conflicts of interest in order to ensure the credibility and integrity of AOAC. Such written disclosure shall be made to any individual or group within the Association which is reviewing a recommendation which the volunteer had a part in formulating and in which the volunteer has a material interest causing an actual or potential conflict of interest.

AOAC requires disclosure of actual or potential conflicts of interest as a condition of active participation in the business of the Association. The burden of disclosure of conflicts of interest or the appearance of conflicts of interest falls upon the volunteer.

A disclosed conflict of interest will not in itself bar an AOAC member from participation in Association activities, but a three-fourths majority of the AOAC group reviewing the issue presenting the conflict must concur by secret ballot that the volunteer's continued participation is necessary and will not unreasonably jeopardize the integrity of the decision-making process.

Employees of AOAC are governed by the provision of the AOAC policy on conflict of interest by staff. If that policy is in disagreement with or mute on matters covered by this policy, the provisions of this policy shall prevail and apply to staff as well.

Illustrations of Conflicts of Interest

1. A volunteer who is serving as a committee member or referee engaged in the evaluation of a method or device; who is also an employee of or receiving a fee from the firm which is manufacturing or distributing the method or device or is an employee of or receiving a fee from a competing firm.

2. A volunteer who is requested to evaluate a proposed method or a related collaborative study in which data are presented that appear detrimental (or favorable) to a product distributed or a position supported by the volunteer's employer.

3. A referee who is conducting a study and evaluating the results of an instrument, a kit, or a piece of equipment which will be provided gratis by the manufacturer or distributor to one or more of the participating laboratories, including his or her own laboratory, at the conclusion of the study.

4. Sponsorship of a collaborative study by an interest (which may include the referee) which stands to profit from the results; such sponsorship usually involving the privilege granted by the investigator to permit the sponsor to review and comment upon the results prior to AOAC evaluation.

5. A volunteer asked to review a manuscript submitted for publication when the manuscript contains information which is critical of a proprietary or other interest of the reviewer.

The foregoing are intended as illustrative and should not be interpreted to be all-inclusive examples of conflicts of interest AOAC volunteers may find themselves involved in.

Do's and Don'ts

Do avoid the appearance as well as the fact of a conflict of interest.

Do make written disclosure of any material interest which may constitute a conflict of interest or the appearance of a conflict of interest.

Do not accept payment or gifts for services rendered as a volunteer of the Association without disclosing such payment or gifts.

Do not vote on any issue before an AOAC decision-making body where you have the appearance of or an actual conflict of interest regarding the recommendation or decision before that body.

Do not participate in an AOAC decision-making body without written disclosure of actual or potential conflicts of interest in the issues before that body.

Do not accept a position of responsibility as an AOAC volunteer, without disclosure, where the discharge of the accepted responsibility will be or may appear to be influenced by proprietary or other conflicting interests.

Procedures

Each volunteer elected or appointed to an AOAC position of responsibility shall be sent, at the time of election or appointment, a copy of this policy and shall be advised of the requirement to adhere to the provisions herein as a condition for active participation in the business of the Association. Each volunteer, at the time of his or her election or appointment, shall indicate, in writing, on a form provided for this purpose by AOAC, that he or she has read and accepts this policy.

Each year, at the spring meeting of the AOAC Board of Directors, the Executive Director shall submit a report certifying the requirements of this policy have been met; including the names and positions of any elected or appointed volunteers who have not at that time indicated in writing that they have accepted the policy.

Anyone with knowledge of specific instances in which the provisions of this policy have not been complied with shall report these instances to the Board of Directors, via the Office of the Executive Director, as soon as discovered.

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Adopted: March 2, 1989
Revised: March 28, 1990
Revised: October 1996

AOAC INTERNATIONAL
ANTITRUST POLICY
STATEMENT AND GUIDELINES

Introduction

It is the policy of AOAC INTERNATIONAL (AOAC) and its members to comply strictly with all laws applicable to AOAC activities. Because AOAC activities frequently involve cooperative undertakings and meetings where competitors may be present, it is important to emphasize the on_going commitment of our members and the Association to full compliance with national and other antitrust laws. This statement is a reminder of that commitment and should be used as a general guide for AOAC and related individual activities and meetings.

Responsibility for Antitrust Compliance

The Association's structure is fashioned and its programs are carried out in conformance with antitrust standards. However, an equal responsibility for antitrust compliance __ which includes avoidance of even an appearance of improper activity __ belongs to the individual. Even the appearance of improper activity must be avoided because the courts have taken the position that actual proof of misconduct is not required under the law. All that is required is whether misconduct can be inferred from the individual's activities.

Employers and AOAC depend on individual good judgment to avoid all discussions and activities which may involve improper subject matter and improper procedures. AOAC staff members work conscientiously to avoid subject matter or discussion which may have unintended implications, and counsel for the Association can provide guidance with regard to these matters. It is important for the individual to realize, however, that the competitive significance of a particular conduct or communication probably is evident only to the individual who is directly involved in such matters.

Antitrust Guidelines

In general, the U.S. antitrust laws seek to preserve a free, competitive economy and trade in the United States and in commerce with foreign countries. Laws in other countries have similar objectives. Competitors (including individuals) may not restrain competition among themselves with reference to the price, quality, or distribution of their products, and they may not act in concert to restrict the competitive capabilities or opportunities of competitors, suppliers, or customers.

Although the Justice Department and Federal Trade Commission generally enforce the U.S. antitrust laws, private parties can bring their own lawsuits.

Penalties for violating the U.S. and other antitrust laws are severe: corporations are subject to heavy fines and injunctive decrees, and may have to pay substantial damage judgments to injured competitors, suppliers, or customers. Individuals are subject to criminal prosecution, and will be punished by fines and imprisonment.
Under current U.S. federal sentencing guidelines, individuals found guilty of bid rigging, price fixing, or market allocation must be sent to jail for at least 4 to 10 months and must pay substantial minimum fines.

Since the individual has an important responsibility in ensuring antitrust compliance in AOAC activities, everyone should read and heed the following guidelines.

1. Don't make any effort to bring about or prevent the standardization of any method or product for the purpose or intent of preventing the manufacture or sale of any method or product not conforming to a specified standard.

2. Don't discuss with competitors your own or the competitors' prices, or anything that might affect prices such as costs, discounts, terms of sale, distribution, volume of production, profit margins, territories, or customers.

3. Don't make announcements or statements at AOAC functions, outside leased exhibit space, about your own prices or those of competitors.

4. Don't disclose to others at meetings or otherwise any competitively sensitive information.

5. Don't attempt to use the Association to restrict the economic activities of any firm or any individual.

6. Don't stay at a meeting where any such price or anti_competitive talk occurs.

7. Do conduct all AOAC business meetings in accordance with AOAC rules. These rules require that an AOAC staff member be present or available, the meeting be conducted by a knowledgeable chair, the agenda be followed, and minutes be kept.

8. Do confer with counsel before raising any topic or making any statement with competitive ramifications.

9. Do send copies of meeting minutes and all AOAC_related correspondence to the staff member involved in the activity.

10. Do alert the AOAC staff to any inaccuracies in proposed or existing methods and statements issued, or to be issued, by AOAC and to any conduct not in conformance with these guidelines.

Conclusion

Compliance with these guidelines involves not only avoidance of antitrust violations, but avoidance of any behavior which might be so construed. Bear in mind, however, that the above antitrust laws are stated in general terms, and that this statement is not a summary of applicable laws. It is intended only to highlight and emphasize the principal antitrust standards which are relevant to AOAC programs. You must, therefore, seek the guidance of either AOAC counsel or your own counsel if antitrust questions arise.

* * * * *

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: March 11, 1991
Revised October 1996

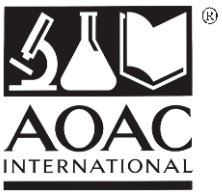
AOAC INTERNATIONAL
POLICY ON THE USE OF THE ASSOCIATION NAME, INITIALS, IDENTIFYING INSIGNIA, LETTERHEAD, AND BUSINESS CARDS

Introduction

The following policy and guidelines for the use of the name, initials, and other identifying insignia of AOAC INTERNATIONAL have been developed in order to protect the reputation, image, legal integrity and property of the Association.

The name of the Association, as stated in its bylaws, is "AOAC INTERNATIONAL". The Association is also known by its initials, AOAC, and by its logo, illustrated below, which incorporates the Association name and a representation of a microscope, book, and flask. The AOAC logo is owned by the Association and is registered with the U.S. Patent and Trademark Office.

The full Association insignia, illustrated below, is comprised of the logo and the tagline, "The Scientific Association Dedicated to Analytical Excellence," shown below. The typeface used is Largo. The AOAC tagline is owned by the Association and is registered with the U.S. Patent and Trademark office.



The Scientific Association Dedicated to Analytical Excellence®

Policy

Policy on the use of the Association's name and logo is established by the AOAC Board of Directors as follows:

> "The Board approves and encourages reference to the Association by name, either as AOAC INTERNATIONAL or as AOAC; or reference to our registered trademark, AOAC®, in appropriate settings to describe our programs, products, etc., in scientific literature and other instances so long as the reference is fair, accurate, complete and truthful and does not indicate or imply unauthorized endorsement of any kind.
>
> The insignia (logo) of AOAC INTERNATIONAL is a registered trade and service mark and shall not be reproduced or used by any person or organization other than the Association, its elected and appointed officers, sections, or committees, without the prior written permission of the Association. Those authorized to use the AOAC INTERNATIONAL insignia shall use it only for the purposes for which permission has been specifically granted.
>
> The name and insignia of the Association shall not be used by any person or organization in any way which indicates, tends to indicate, or implies AOAC official endorsement of any product, service, program, company, organization, event or person, endorsement of which, has not been authorized by the Association, or which suggests that membership in the Association is available to any organization."

The Executive Director, in accordance with the above stated policy, is authorized to process, approve, fix rules, and make available materials containing the Association name and insignia.

It should be noted that neither the Association's name nor its insignia nor part of its insignia may be incorporated into any personal, company, organization, or any other stationery other than that of the Association; nor may any statement be included in the printed portion of such stationery which states or implies that an individual, company, or other organization is a Member of the Association.

Instructions

1. Reproduction or use of the Association name or insignia requires prior approval by the Executive Director or his designate.

2. Association insignia should not be altered in any manner without approval of the Executive Director or his designate, except to be enlarged or reduced in their entirety.

3. Artwork for reproducing the Association name or insignia, including those incorporating approved alterations, will be provided on request to those authorized to use them (make such requests to the AOAC Marketing Department). Examples of the types of alterations that would be approved are inclusion of a section name in or the addition of an officer's name and address to the letterhead insignia.

4. When the Association name is used without other text as a heading, it should, when possible, be set in the Largo typeface.

5. Although other colors may be used, AOAC blue, PMS 287, is the preferred color when printing the AOAC insignia, especially in formal and official documents. It is, of course, often necessary and acceptable to reproduce the insignia in black.

6. Do not print one part of the logo or insignia in one color and other parts in another color.

7. The letterhead of AOAC INTERNATIONAL shall not be used by any person or organization other than the Association, its elected and appointed officers, staff, sections, or committees; except by special permission.

   Correspondence of AOAC official business should be conducted using AOAC letterhead. However, those authorized to use AOAC letterhead shall use it for official AOAC business only.

   Copies of all correspondence using AOAC letterhead or conducting AOAC official business, whether on AOAC letterhead or not, must be sent to the appropriate office at AOAC headquarters.

8. AOAC INTERNATIONAL business cards shall not be used by any person or organization other than the Association, its staff, and elected officials, except by special permission.

   Those authorized to use AOAC business cards shall use them for official AOAC business only and shall not represent themselves as having authority to bind the Association beyond that authorized.

Sanctions

1. Upon learning of any violation of the above policy, the Executive Director or a designate will notify the individual or organization that they are in violation of AOAC policy and will ask them to refrain from further misuse of the AOAC name or insignia.

2. If the misuse is by an Individual Member or Sustaining Member of the Association, and the misuse continues after notification, the Board of Directors will take appropriate action.

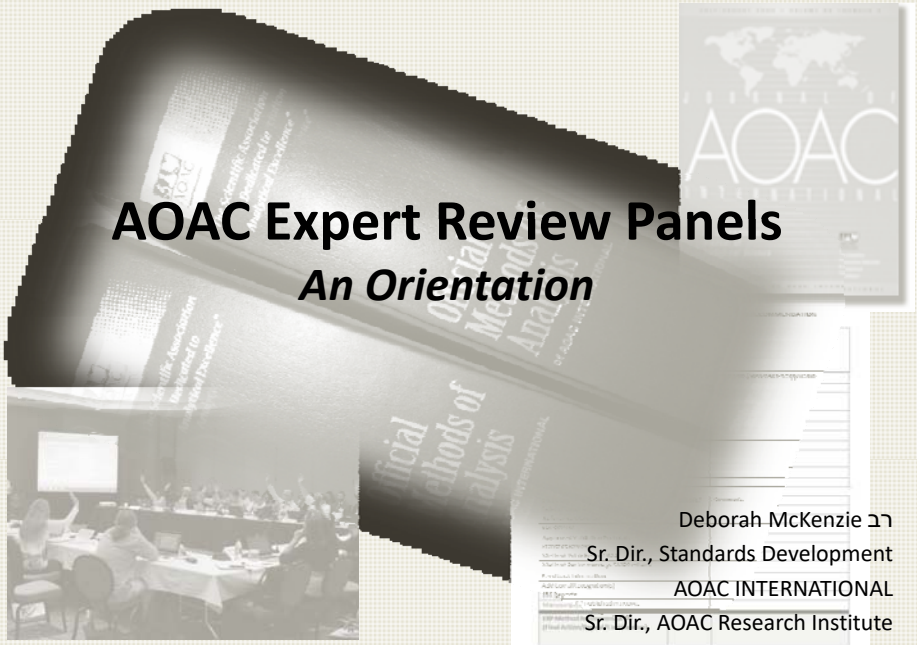
3. If continued misuse is by a nonmember of the Association or if a member continues misuse in spite of notification and Board action, ultimately, the Association will take legal action to protect its property, legal integrity, reputation, and image.

* * * * * *

Adopted by the AOAC Board of Directors: September 24, 1989
Revised: June 13, 1991; February 26, 1992; March 21, 1995; October 1996

AOAC Expert Review Panels

An Orientation



Deborah McKenzie רב
Sr. Dir., Standards Development
AOAC INTERNATIONAL
Sr. Dir., AOAC Research Institute
Staff Liaison - Official Methods Board

AOAC Method Approval Programs

AOAC INTERNATIONAL

- Administers *Official Methods*^{SM} program based on AOAC standards development activity
- Adoption of methods as *Official Methods* is contingent upon standards development activities
- No application fee required to submit methods in response to Call for Methods
- Method submissions coincide with standards development activities

AOAC Research Institute

- Administers *Official Methods*^{SM} program based on individual submissions
- Sole source and individual method submissions
- Application fee required

AOAC Policies & Procedures

- Policy on Antitrust
- Policy on Use of Association Name, Identifying Insignia, Letterhead, Business Cards
- Policy on Volunteer Conflict of Interest
- Expert Review Panel Policies and Procedures
- OMA Appendix G

Policies and Procedures for Adoption of *Official Methods of Analysis*

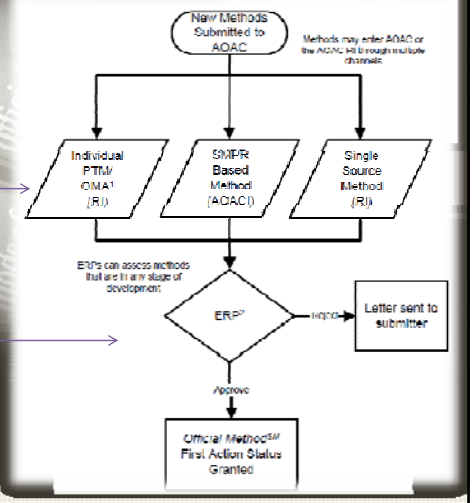
- *OMA, Appendix G: Procedures and Guidelines for the Use of AOAC Voluntary Consensus Standards to Evaluate Characteristics of a Method of Analysis*
  - *Expert Review Panels, Official Methods Board, First and Final Action Official Methods*
  - *First Action to Final Action Methods: Guidance for AOAC Expert Review Panels*
- *Expert Review Panels – Policies and Procedures*
- *Appendix F: Guidelines for Standard Method Performance Requirements*
- *OMA, About the AOAC Official Methods℠ Program*

Road to First Action OMA Status

Terms:
- PTM – *Performance Tested Methods℠*
- RI – Research Institute
- ERP – Expert Review Panel
- OMB – Official Methods Board
- SP – Stakeholder Panel
- SMPR – Standard Method Performance Requirement

Three modes of entry and (program administration)

Expert Review Panels will review all methods for all three modes of entry.



Note: Appeals process always available; see Alternative Pathway Guidelines for appeals process.

1 PTM certification previously issued, PTM reviewers will be ERP members
2 Unless otherwise provided for under a contractual agreement, AOAC will regularly convene ERPs twice a year: once during the Mid-Year Meeting and again during the Annual Meeting

Road to Final Action OMA Status

Method reproducibility must be demonstrated before Final Action consideration.

ERP determines if sufficient evidence merits a recommendation for Final Action status or repeal.

•Only the OMB promotes a method to "Final Action" status or repeal the method.

•Methods that did not meet the bar would be repealed.

•Same for all method submissions

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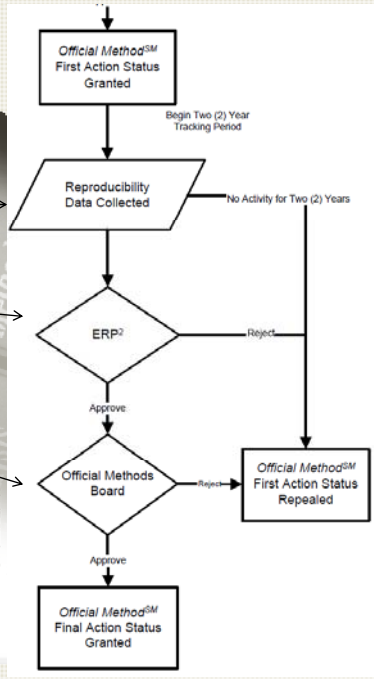

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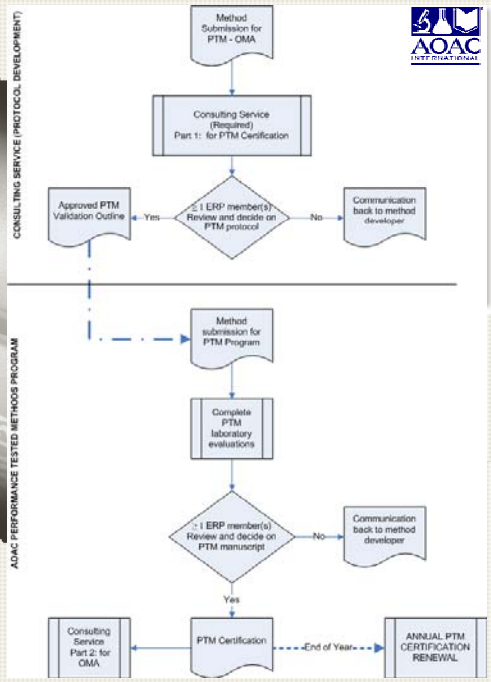
PTM Overview for PTM-OMA Harmonized Process

- Administered by the Research Institute in 2003.
- Well established and streamlined
- Original approved by consensus with the OAs, OMB, RI Board of Directors and AOAC INTERNATIONAL Board of Directors.
- ERP may be formed during Consulting Service.
- Criterion for OMA: manufacturer's method claims.



AOAC Method Approval Programs

Official Methods of Analysis[SM] (OMA)

- AOAC's premiere methods program
- Approved methods
  - published in the *Official Methods of Analysis of AOAC INTERNATIONAL* (print and online)
  - Manuscripts published in the *Journal of AOAC INTERNATIONAL*
  - *First Action and Final Action status*

Performance Tested Methods[SM] (PTM)

- AOAC's method certification program
- Certified methods
  - Commercial/proprietary rapid methods (test kits)
  - Certifications published on AOAC website
  - Manuscripts published in the Journal of AOAC INTERNATIONAL
  - Method developers licensed to use certification mark
  - Annual review & recertification

Qualifications for ERP Membership

Candidate must meet one of the following:

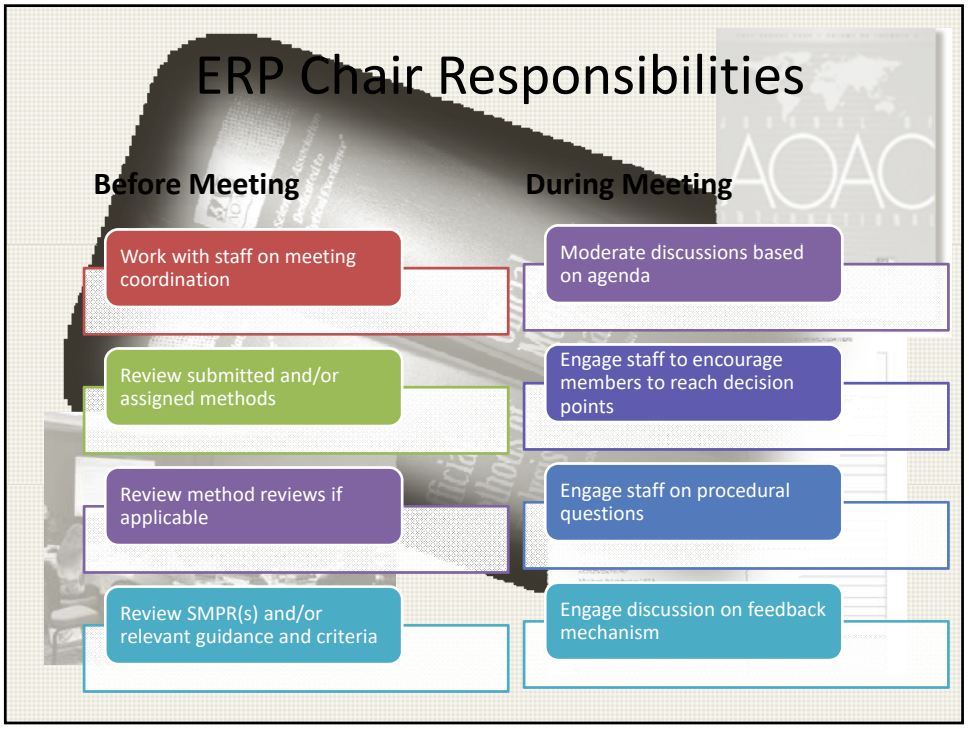
- Demonstrated knowledge in the appropriate scientific disciplines.
- Demonstrated knowledge regarding data relevant to adequate method performance.
- Demonstrated knowledge of practical application of analytical methods to bona fide diagnostic requirements.

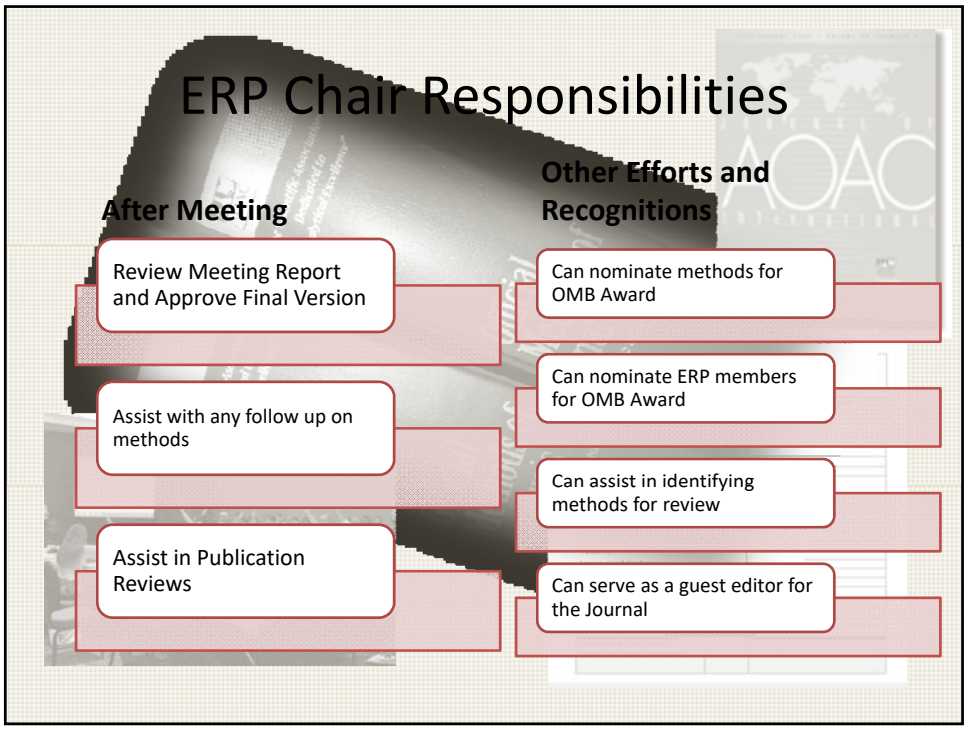
Candidate application package includes:

- Statement of Expertise
- Current Abridged CV or Resume

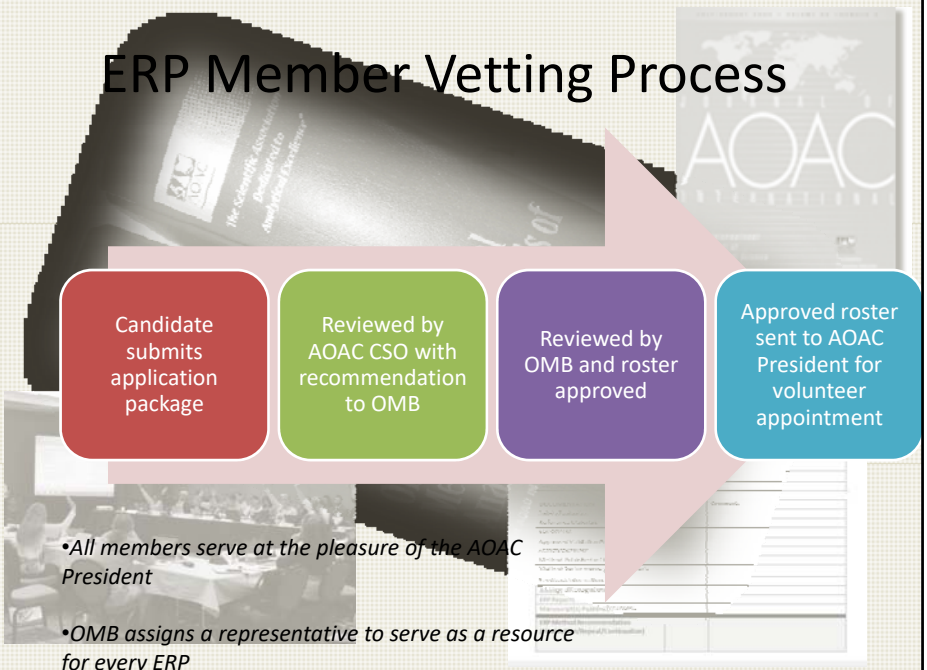
Experts and Methods

- AOAC issues
  - Call for Methods *(Stakeholder affiliated methods)*
  - Call for Experts
- Sole Source/Individual Method Submissions
  - Applications to Research Institute


ERP Chair Responsibilities
Before Meeting
Work with staff on meeting coordination
Review submitted and/or assigned methods
Review method reviews if applicable
Review SMPR(s) and/or relevant guidance and criteria
During Meeting
Moderate discussions based on agenda
Engage staff to encourage members to reach decision points
Engage staff on procedural questions
Engage discussion on feedback mechanism


ERP Chair Responsibilities
After Meeting
Review Meeting Report and Approve Final Version
Assist with any follow up on methods
Assist in Publication Reviews
Other Efforts and Recognitions
Can nominate methods for OMB Award
Can nominate ERP members for OMB Award
Can assist in identifying methods for review
Can serve as a guest editor for the Journal

ERP Member Vetting Process



- *All members serve at the pleasure of the AOAC President*
- *OMB assigns a representative to serve as a resource for every ERP*

Candidate Method Reviews

- In your judgment, does the method sufficiently meet the Standard Method Performance Requirements (SMPR) or community-based guidance?
- In your judgment, is the method scientifically sound and can be followed?
- In your judgment, what are the strengths and weaknesses of the method?
- In your judgment, how do the weaknesses weigh in your recommendation for the method?
- In your judgment, will the method serve well the stakeholder community that will use the method?
- In your judgment, what additional information may be needed to further support the method meeting the SMPR or community-based guidance?
- Members of both Committee on Safety and Committee on Statistics serve as advisory resources for all ERPs

ERP Meetings

- ERPs will meet in person at a minimum of twice a year and up to four times per year:
  - AOAC Mid-Year meeting (DC metro area)
  - AOAC Annual Meeting.
  - 2 additional designated times for proprietary method Organziational Affiliates
- At the ERP meeting:
  - Reviews will be presented and a primary or secondary reviewer can make a motion/recommendation to the ERP whether or not to adopt the method as First Action OMA.
  - ERP discusses the method.
  - ERP renders a decision on First Action status.
  - ERP renders decisions on modifications to First Action methods only.
- If the method is adopted
  - ERP decides on what additional information is needed to recommend the method for Final Action status

ERP Meetings

Quorum

Presence of 7 vetted ERP members

OR

Presence of 2/3 vetted ERP members

WHICHEVER IS GREATER

IF NO QUORUM, NO OFFICIAL MEETING

Method Review Overview

- Method authors may be invited to make a presentation on their method
- REVIEWERS PRESENT THEIR REVIEWS AND MAY INITIATE A MOTION TO ADOPT THE METHOD IF THEY CHOOSE
  - Chair recognizes each reviewer
  - Primary and secondary reviews are presented.
    - If in favor, they may make and second a motion to adopt or not adopt the method
    - Chair can then entertain discussion on the method
    - Chair can call for a vote once deliberation is complete

Consensus – First Action Adoption

- First Action Official Methods status is granted:
- Method must be adopted by unanimous decision of ERP on first ballot, if not unanimous, negative votes must delineate scientific reasons.
- Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration.
- Method becomes First Action on the date when ERP decision is made.

Consensus – First Action to Final Action

- The ERP may then reach consensus on any additional information that it needs to review to be able to make a recommendation for Final Action *Official Methods* status.
- This is a separate motion.

Road to First Action OMA Status

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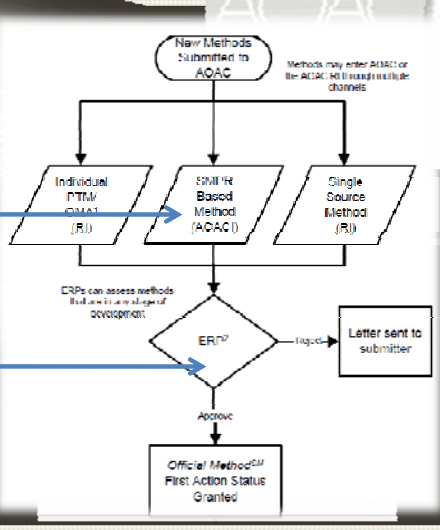


Note: Appeals process always available; see Alternative Pathway Guidelines for appeals process.

1 PTM certification previously issued, PTM reviewers will be ERP members
2 Unless otherwise provided for under a contractual agreement, AOAC will regularly convene ERPs twice a year: once during the Mid-Year Meeting and again during the Annual Meeting

ERP Meetings – Review for First Action

METHOD AUTHOR: present any method and any resulting changes to the method since submission for review, summary of SLV and/or reproducibility evaluation, any recognitions (from AOAC or external) and, final draft of method proposed for decision

ERP CHAIR & MEMBERS: present reviews and discuss any resulting issues or questions on the method, review and agree upon final draft of method proposed for decision, and chair calls for ERP decision in accordance to procedures.

CONSENSUS: Method must be adopted by unanimous decision of ERP on first ballot. If not unanimous, negative votes must delineate scientific reasons. Negative voter(s) can be overridden by 2/3 of non-negative voting ERP members after due consideration.

Abstentions do not count towards vote; in case of multiple abstentions the results will need to be evaluated. Staff will monitor and record consensus voting.

STAFF: Will organize and coordinate meeting, record ERP actions and decisions, draft ERP report and distribute after chair approval, work with chair and OMB liaison to complete checklist and assemble recommendation package for OMB.

ERP Methods Review & Approval

Methods should be scientifically sound with demonstrating that it will meet the needs of those using the method (evidenced by meeting the standard, or other acceptance criteria)

ERPs have approved methods with evidence of high potential to First Action and request additional work or support be submitted for review prior to ERP convening to recommend an action to OMB

OMB requires a justification or rationale for methods that are deemed acceptable and adopted but may not fully meet the standard set or acceptance criteria.

OMB Expectations for First Action

- Safety review needed prior to First Action status
- SLV type of supporting information available per the SMPR
  - Applicability, Method Performance Requirements Table, System Suitability, Reference Materials, and Validation Guidance
- Comparison to SMPR
  - Documented method performance versus a SMPR
  - Document reasons for acceptability if method does not meet the SMPR

Publication of First Action Methods

- Any approved method(s) along with supporting manuscript(s) and documentation sent to AOAC Publications after the meeting.
  1. Method incorporating ERP revisions (preferably in AOAC Format)
  2. Method Manuscript incorporating specified ERP revisions (in AOAC Format)
  3. Signed AOAC Copyright Authorization form

NO OMA NUMBER ASSIGNED UNTIL ALL DOCUMENTATION SUBMITTED

- Method and method manuscript prepared for publication in the *Official Methods of Analysis of AOAC INTERNATIONAL* and in Journal of AOAC INTERNATIONAL
- Updates on methods approved or status changes are published in the Inside Laboratory Management magazine and on the AOAC website

ERP Meetings – Method Tracking

METHOD AUTHOR: present any method feedback obtained and any resulting changes to the method, any reproducibility information, any implemented ERP recommendations, final draft of method proposed for decision

ERP MEMBERS: present any method feedback obtained and discuss any resulting changes to the method, any reproducibility information, any implemented ERP recommendations, review and agree upon final draft of method proposed for decision, and make a recommendation to OMB.

CONSENSUS: 2/3 vote in favor of a motion. Abstentions do not count towards vote; in case of multiple abstentions. Staff will monitor and record consensus voting.

STAFF: Will organize and coordinate meeting, record ERP actions and decisions, draft ERP report and distribute after chair approval, work with chair and OMB liaison to complete checklist and assemble recommendation package for OMB.

OMA, Appendix G

ERP to recommend Method to Official Final Action Status to the OMB.

OMB Liaison Assigned to ERP

ERP Recommendation to OMB

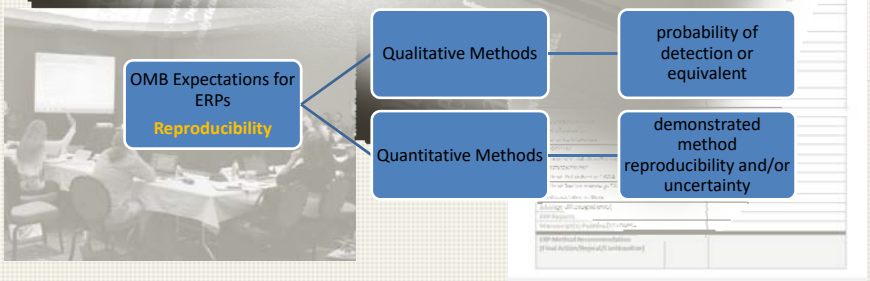
Checklist for First Action Recommendations

Documents supporting ERP Recommendations

OMA, Appendix G

Further data indicative of adequate method reproducibility (between laboratory) performance to be collected. Data may be collected via a collaborative study or by proficiency or other testing data of similar magnitude.

- ERP is looking to verify if method reproducibility has been appropriately assessed and satisfactorily demonstrated



OMA, Appendix G

Two years maximum transition time (additional year(s) if ERP determines a relevant collaborative study or proficiency or other data collection is in progress).

2 yr tracking of method

- ERP verification of any changes to the method
- ERP recommendations implemented successfully
- ERP evaluation of any feedback on method and its performance

ERP Recommendations

- Move method to Final Action OMA status
- Repeal method from OMA
- Continuance of First Action OMA status

OMA, Appendix G

Method removed from Official First Action and OMA if no evidence of method use or if no data indicative of adequate method reproducibility available at the end of the transition time.

First Action OMA Tracking

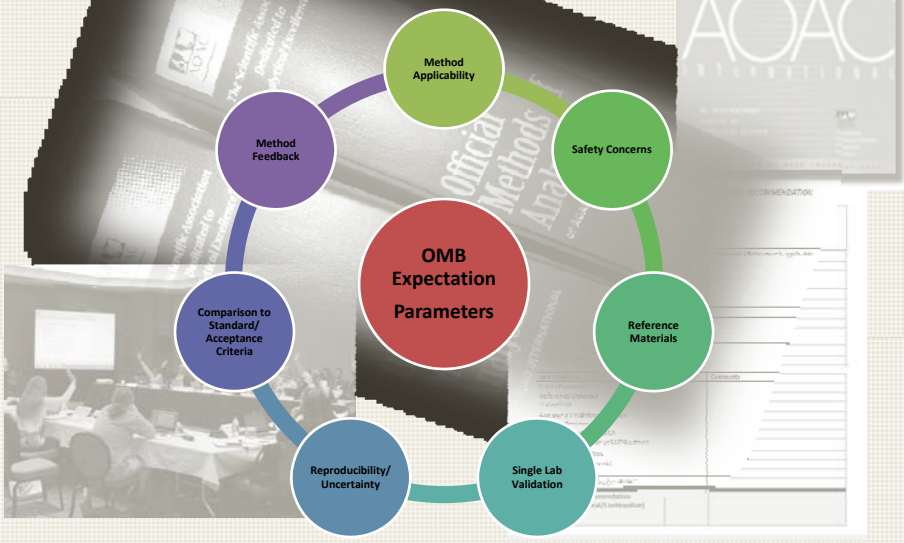
- Tracking period is ≤ 2 years and begins on the date of the ERP's decision to adopt a method for OMA First Action status.

No Use in 2 Years

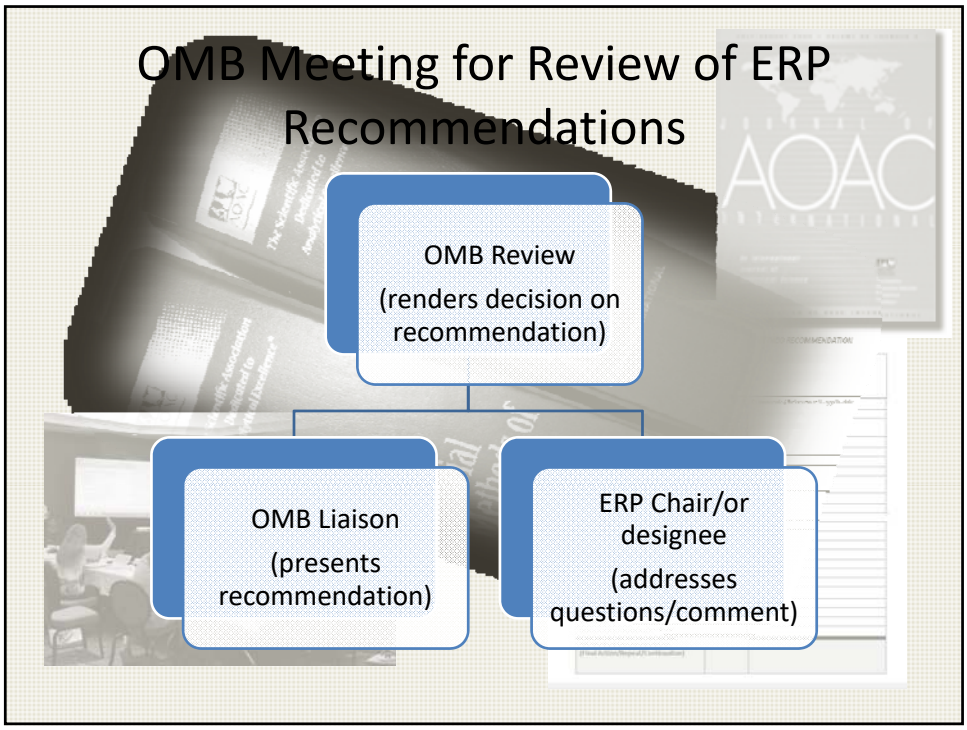
- Repeal from OMA

OMA, Appendix G

First Action to Final Action Methods: Guidance for AOAC Expert Review Panels




Documentation Needed
Method Safety Evaluation
Reference Materials
Evidence of Single Laboratory Validation or equivalent
Evidence of Reproducibility Assessment
Published First Action OMA
Method Performance versus SMPR or acceptance criteria
Final draft of First Action OMA to be considered for status update
Rationale or Justification for Repeal or Continuance of First Action OMA

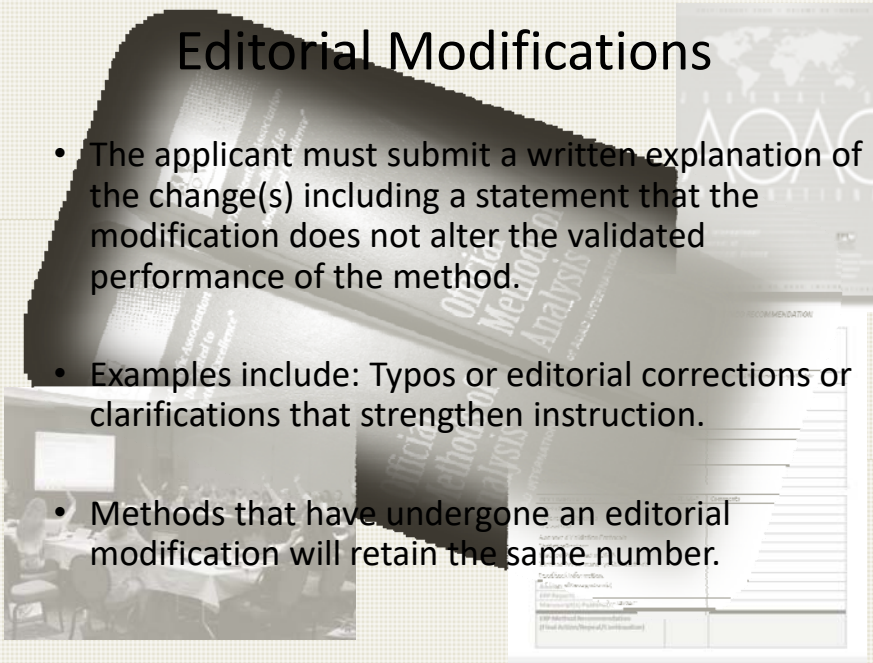

OMB Meeting for Review of ERP Recommendations
OMB Review
(renders decision on recommendation)
OMB Liaison
(presents recommendation)
ERP Chair/or designee
(addresses questions/comment)

Modifications to Official Methods

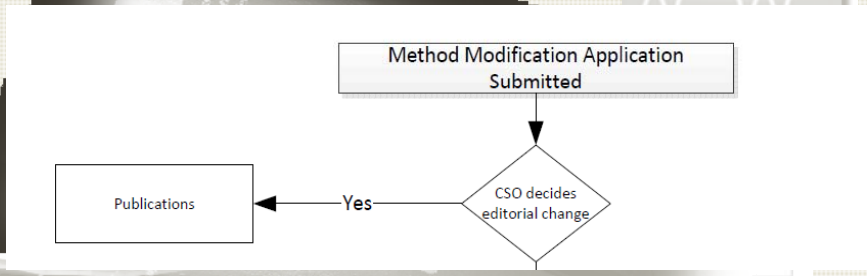
- Types of Modifications
  - Editorial
  - Major
  - Minor
- Applicable to First Action and Final Action OMA
- Relevant to all ERPs

Editorial Modifications

- The applicant must submit a written explanation of the change(s) including a statement that the modification does not alter the validated performance of the method.
- Examples include: Typos or editorial corrections or clarifications that strengthen instruction.
- Methods that have undergone an editorial modification will retain the same number.



Editorial Changes



- Editorial changes to methods only require AOAC staff review and the change is made to the OMA with changes noted in next printed edition of OMA.
- A list of the methods with editorial modifications will be published in *Inside Laboratory Management and on* the Website.

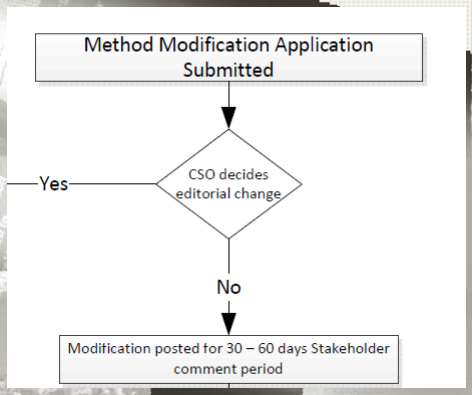
Minor Modifications

- Results in no changes to the current validated performance. There is no significant effect to the results. The method will retain the original number.
- Supporting data to justify the proposed modification must be submitted. Equivalency data is required unless adequate Justification to exclude this data is provided.
- Examples include: Reagent change, a change in a column or consumables that do not impact the validated method performance.

Major Modifications

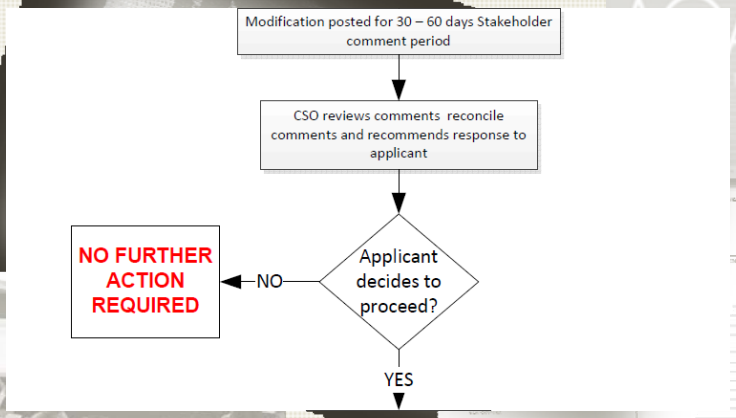
- Results in a change to the current validated performance of the method.
- This level of modification will result in a new method as part of AOAC standards development and will receive a new method number.
- Examples include: significant change to the technology, sample preparation, or chemistry.

Minor & Major Modifications



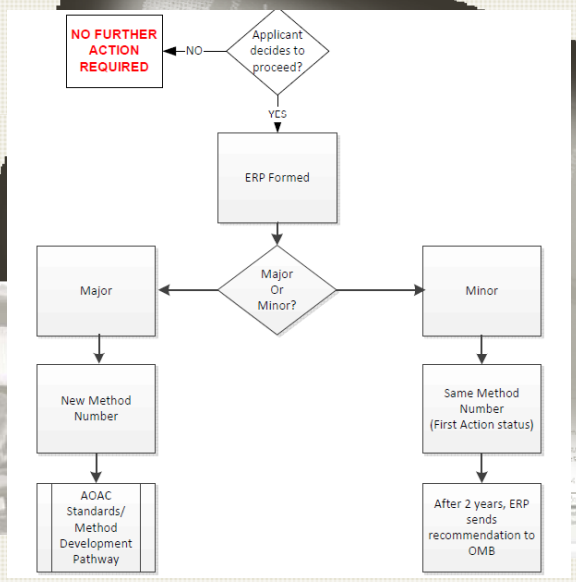
Based on AOAC staff review, a public comment period for the proposed modification is required.

Applicant Options



- Following the comment period, any comments are reconciled and recommends a response to the applicant.
- The applicant can decide to proceed based on the reconciled comments

Pathways for Minor & Major Modification



If applicant decides to proceed, an ERP is formed

- Level of modification determined by ERP
- Applies to modifications of First Action and Final Action methods

Documentation and Communication

- AOAC carefully documents the actions of Stakeholder Panel and the Working Groups
- AOAC will prepare summaries of the meetings
  - Communicate summaries to the stakeholders
  - Publish summaries in the *Referee* section of AOAC's *Inside Laboratory Management*
- AOAC publishes its voluntary consensus standards and Official Methods
  - *Official Methods of Analysis of AOAC INTERNATIONAL*
  - *Journal of AOAC INTERNATIONAL*
- AOAC publishes the status of standards and methods in the Referee section of AOAC's *Inside Laboratory Management*

Roles and Responsibilities

AOAC Official Methods Board

- Vet and approve stakeholder panel chair & voting members
- Vet and approve ERP membership and AOAC Experts
- Render decisions on status of First Action methods (Final Action, repeal, etc…)
- Assign a liaison to each stakeholder panel and ERP
- Coordinate OMB Awards

AOAC Expert Review Panels

- Review methods and meet in person to render decisions on methods for First Action Official Methods℠ status.
- Track First Action Official Methods℠ and modify, if necessary
- Recommend First Action methods after 2 years or less to OMB for Final Action, continuance, or Repeal
- Participate in Consulting Service and PTM reviews for OMA and harmonized PTM and harmonized OMA method studies

AOAC Experts

- Review and approve PTM validation testing protocol documentation
- Peer review of PTM validation manuscript and supporting documentation

AOAC Research Institute - PTM Expert Reviewers

- Peer Review of PTM validation manuscripts and supporting documentation

AOAC Research Institute Independent Laboratories

- Conduct independent evaluation of candidate method using AOAC approved testing protocols

AOAC Stakeholder Panels

- Develop voluntary consensus standards
- Assign working groups to draft standards method performance requirements
- Voting members demonstrate consensus on behalf of stakeholders

AOAC Staff

- Coordinate method reviews and method approval activities
- Coordinate OMB meetings
- Provide trainings and orientations
- Maintain website and communication
- Document and publish actions and decisions
- Coordinate standards development activities
- Publish standards and methods

AOAC Research Institute Technical Consultants

- Draft validation protocols in Consulting Service for assigned methods
- Facilitate PTM evaluation of assigned candidate methods
- Facilitate comments/responses for assigned OMA reviews


Questions?
Thank you
☺
AOAC
Official Methods of Analysis

Official Methods of Analysis[SM] (OMA) Expert Review Panel
MEETING AND METHOD REVIEW GUIDANCE

The AOAC Research Institute administers AOAC INTERNATIONAL's premier methods program, the AOAC *Official Methods of Analysis*[SM] (OMA). The program evaluates chemistry, microbiology, and molecular biology methods. It also evaluates traditional benchtop methods, instrumental methods, and proprietary, commercial, and/or alternative methods and relies on gathering the experts to develop voluntary consensus standards, followed by collective expert judgment of methods using the adopted standards. The *Official Methods of Analysis of AOAC INTERNATIONAL* is deemed to be highly credible and defensible.

All Expert Review Panel (ERP) members are vetted by the AOAC Official Methods Board (OMB) and serve at the pleasure of the President of AOAC INTERNATIONAL. In accordance to the AOAC Expert Review Panel Member and Chair Volunteer Role Description all Expert Review Panel members are expected to 1) serve with the highest integrity, 2) perform duties and method reviews, and 3) adhere to review timelines and deadlines.

To assist the ERP Chair and its members, please note the following in preparation for Expert Review Panel meetings and method reviews.

Pre-Meeting Requirements

1. Confirm availability and plan to be present to ensure a quorum of the ERP.
   (Please refer to page 25, Quorum Guidelines, Expert Review Panel Information Packet)
2. Ensure that your laptop, CPU or mobile device can access online web documentation.
3. Be prepared for the meeting by reviewing all relevant meeting materials and method documentation.

In-Person Meeting and Teleconference Conduct

1. Arrive on time.
2. Advise the Chair and ERP members of any potential Conflicts of Interest at the beginning of the meeting.
3. Participation is required from all members of the ERP. All members have been deemed experts in the specific subject matter areas.
4. The ERP Chair will moderate the meeting to ensure that decisions can be made in a timely manner.
5. Follow Robert's Rules of Order for Motions.
6. Speak loud, clear, and concise so that all members may hear and understand your point of view.
7. Due to the openness of our meetings, it is imperative that all members communicate in a respectful manner and tone.
8. Refrain from disruptive behavior. Always allow one member to speak at a time. Please do not interrupt.
9. Please note that all methods reviewed and decisions made during the Expert Review Panel process are considered confidential and should not be discussed unless during an Expert Review Panel meeting to ensure transparency.

Reviewing Methods

Prior to the Expert Review Panel meeting, ERP members are required to conduct method reviews. All methods are reviewed under the following criteria, technical evaluation, general comments, editorial criteria, and recommendation status. These methods are being reviewed against their collaborative study protocols as provided in the supplemental documentation. *Note: The method author(s) will be present during the Expert Review Panel session to answer any questions.*

Reviewing Methods (Cont'd)

- Reviewers shall conduct in-depth review of method and any supporting information.
- In-depth reviews are completed electronically via the method review form. The method review form must be completed and submitted by the deadline date as provided.
- All reviews will be discussed during the Expert Review Panel meeting.
- Any ERP member can make the motion to adopt or not to adopt the method.
- If the method is adopted for AOAC First Action status, Expert Review Panel members must track and present feedback on assigned First Action *Official Methods*.
- Recommend additional feedback or information for Final Action consideratio**n.**

Here are some questions to consider during your review based on your scientific judgment:

1. Does the method sufficiently follow the collaborative study protocol?
2. Is the method scientifically sound and can be followed?
3. What are the strengths and weaknesses of the method?
4. How do the weaknesses weigh in your recommendation for the method?
5. Will the method serve the community that will use the method?
6. What additional information may be needed to further support the method?
7. Can this method be considered for AOAC First Action OMA status?

Reaching Consensus during Expert Review Panel Meeting

1. Make your Motion.
2. Allow another member to Second the Motion.
3. The Chair will state the motion and offer the ERP an option to discuss the motion.
4. The Chair will call a vote once deliberations are complete.
5. Methods must be adopted by unanimous decision of ERP on first ballot, if not unanimous, negative votes must delineate scientific reasons. Negative voter(s) can be overridden by 2/3 of voting ERP members after due consideration.
6. All other motions will require 2/3 majority for vote to carry.

Author Response to Reviewers:

1. Table 6, which is the comparison of the results of certified and consensus values for all elements included in this study, has been updated to include a bias for both the NIST-SRM 695 and Magruder 2009-06. Please see the attachment for corrections.

2. Table 7, which is demonstrates the method precision and comparability to the 2006.03 method using some of the original materials and original included elements, has been updated using the statistician's suggestion of multiplying the found Horrat value by two. Please see the attachment for corrections. Please see the attachment for corrections.

3. Table 8, which is the method precision for the proposed included elements (Ca, Cu, Fe, Mg, and Mn) using some of the original study materials, has been updated using the suggestion from the statistician of multiplying the found Horrat value by two. Please see the attachment for corrections.

4. The author recommends a change to the 2006.03 method for the removal from the scope of arsenic, selenium, and lead due to poor statistics. However, at the time of the publication, it was the best method available using common equipment found in both state regulatory and commercial laboratories. The improvement of the added hydrochloric acid to the method will provide adequate recovery, reproducibility, and trueness of the results lacking in the nitric only method.

5. Spike method: This method will be changed to a new method and it is not addressed at this time.

6. Please see the attachment labeled: "Fertilizer Subgroup Metals Statement Rev_071516_OMAMAN-28-AOAC 2006.03" for further explanations regarding this method.

	NIST SRM 695				Magruder 2009-06			
Element	Certified	Mean	Recovery, %	Bias	Concensus, ICP	Mean	Recovery, %	Bias
As, mg/kg	200 ± 5	199.9	100.0	0.1	330.58 ± 20.55	358.45	108.43	27.87
Cd, mg/kg	16.9 ± 0.2	17.1	111.2	0.2	343.55 ± 19.70	348.14	101.33	4.59
Co, mg/kg	65.3 ± 2.4	61.7	103.9	3.6	945.97 ± 53.68	959.33	101.41	13.36
Cr, mg/kg	244 ± 6	226.4	92.8	17.6	111.68 ± 11.16	127.93	114.55	16.25
Mo, mg/kg	20.0 ± 0.3	19.5	107.3	0.5	17.80 ± 2.70	18.10	101.66	0.30
Ni, mg/kg	135 ± 2	127.6	94.5	7.4	1135.8 ± 81.32	1117.33	98.37	18.47
Pb, mg/kg	276 ± 17	284.9	103.2	8.9	3688.5 ± 1852.4	4869.59	132.02	1181.1
Se, mg/kg	2.1 ± 0.1*	1.6	74.6	0.5	116.46 ± 8.33	110.56	94.93	5.90
Ca, %	2.26 ± 0.04	2.3	102.5	0.0	1.78 ± 0.12	1.79	100.43	0.01
Cu, ppm	1225 ± 9	1214.4	99.1	10.6	334 ± 38	339.69	101.70	5.69
Fe, %	3.99 ± 0.08	4.0	99.7	0.0	2.03 ± 1.02	3.03	149.03	1.00
Mg, %	1.79 ± 0.05	1.8	98.2	0.0	0.18 ± .12	0.19	105.89	0.01
Mn, %	0.305 ± 0.005	0.3	101.9	0.0	0.153 ± 0.013	0.18	115.54	0.02
Zn, %	0.325 ± 0.005	0.3	97.7	0.0	0.165 ± 0.11	0.16	99.25	0.00

*Reference Value

Table 6. Comparison of results of certified and consensus values with bias included.

Table 7. Method precision and comparability to 2006.03, revised.

	Proposed method (n=3)		Collaborative study 2006.03 results			
Material	Avg., mg/kg	RSD, %	Avg., mg/kg	RSDr, %	Recovery, %	Horrat(r)
			As			
A	41.49	1.86	22.15	42.93	187.32	0.58
B	478.89	1.39	263.2	47.96	181.95	0.62
C	6.89	3.05	4.87	63.12	141.42	0.72
D	5917.76	3.43	4945	6.09	119.67	2.24
E	2953.58	0.62	2432	10.56	121.45	0.36
F	10.36	2.10	9.75	41.73	106.26	0.54
G	22.32	0.35	22.43	9.36	99.49	0.10
H	2.92	3.32	2.36	17.6	123.63	0.70
I	11.95	1.77	13.04	13.27	91.63	0.46
J	189.46	2.23	185.45	4.27	102.16	0.86
K	168.49	2.23	175.28	7.84	96.13	0.86
L	bdl	NA	NA	NA	NA	NA
M	9.31	1.45	7.35	53.17	126.64	0.36
N	17.41	1.82	12.74	15.74	136.62	0.50
O	3.77	4.95	4.16	45.14	90.66	1.08
P	60.72	3.73	47.83	2.22	126.94	1.22
Cd						
A	2.40	3.92	2.25	39.14	106.78	0.80
B	5.29	7.83	7.56	19.65	69.97	1.78
C	22.07	1.79	21.28	0.61	103.72	0.50
D	44.91	4.04	36.64	3.2	122.56	1.28
E	27.86	6.51	22.58	4.25	123.4	1.90
F	235.39	0.76	214.6	3.06	109.69	0.30
G	28.73	1.61	26.69	5.79	107.65	0.48
H	63.58	0.9	55.29	1.25	115.00	0.30
I	bdl	NA	NA	NA	NA	NA
J	16.65	0.44	15.52	2.77	107.26	0.12
K	66.05	2.29	64.04	2.95	103.14	0.76
L	bdl	NA	NA	NA	NA	NA
M	4.32	9.64	4.19	3.18	103.17	2.14
N	bdl	NA	NA	NA	NA	NA
O	bdl	NA	NA	NA	NA	NA
P	0.52	20.46	0.57	66.74	92.02	1.88
			Co			
A	119.71	2.69	97.75	4.12	122.47	0.98

B	212.27	0.89	195.6	9.34	108.52	0.36
C	bdl	NA	NA	NA	NA	NA
D	19.8	0.39	17.33	4.00	114.25	0.10
E	26.5	1.28	23.01	3.53	115.15	0.38
F	9.05	1.14	8.91	2.94	101.57	0.28
G	bdl	NA	NA	NA	NA	NA
H	bdl	NA	NA	NA	NA	NA
I	bdl	NA	NA	NA	NA	NA
J	59.57	0.39	45.20	8.52	131.79	0.16
K	545.71	3.30	532.78	2.45	102.43	1.28
L	bdl	NA	NA	NA	NA	NA
M	22.54	4.39	21.25	4.98	106.08	1.52
N	bdl	NA	NA	NA	NA	NA
O	bdl	NA	NA	NA	NA	NA
P	13.65	2.12	10.67	9.14	127.93	0.56
			Cr			
A	892.48	2.46	731.5	10.09	122.01	2.34
B	461.2	1.26	396.99	14.2	116.17	1.10
C	172.14	0.47	159.5	1.31	107.93	0.36
D	45.39	0.66	38.25	3.66	118.67	0.40
E	122.57	1.23	101.15	2.29	121.18	0.88
F	586.13	0.83	566.16	8.00	103.53	0.76
G	302.41	1.00	281.91	2.88	107.27	0.84
H	380.12	0.32	341.28	3.05	111.38	0.28
I	18.31	0.71	18.11	1.26	101.09	0.38
J	219.62	0.14	164.4	10.38	133.59	0.10
K	189.16	1.89	169.49	2.76	111.6	1.44
L	6.41	1.97	5.84	5.52	109.78	0.46
M	129.29	2.29	115.55	2.69	111.89	1.66
N	6305.07	2.55	5980.93	0.99	105.42	3.34
O	120.89	1.36	108.85	6.75	111.88	0.98
P	146.07	2.83	134.77	7.08	108.38	2.10
			Mo			
A	109.17	0.90	69.16	7.35	157.85	0.6
B	156.58	2.47	116.69	18.28	134.18	1.78
C	4.84	2.5	3.89	10.04	124.4	1.08
D	3.72	3.19	2.73	21.89	136.22	1.32
E	6.88	6.27	4.39	22.27	156.79	1.40
F	20.48	1.26	18.47	1.68	110.91	0.68
G	4.41	1.47	4.00	12.49	110.31	0.64

H	13.69	0.79	11.74	4.75	116.61	0.40
I	bdl	NA	NA	NA	NA	NA
J	19.39	0.36	13.21	7.94	146.76	0.18
K	44.68	2.1	42.88	5.23	104.19	1.30
L	bdl	NA	NA	NA	NA	NA
M	14.7	1.93	11.53	15.03	127.53	0.98
N	9.31	1.92	7.83	7.22	118.88	0.46
O	bdl	NA	NA	NA	NA	NA
P	15.53	0.76	12.44	14.46	124.83	0.40
			Ni			
A	384.29	0.57	331.92	3.94	115.78	0.70
B	330.3	3.66	295.83	18.02	111.65	1.10
C	30.10	3.63	26.60	3.55	113.14	0.74
D	bdl	NA	NA	NA	NA	NA
E	39.14	3.35	36.39	5.75	107.57	0.74
F	296.16	0.38	279.34	0.93	106.02	0.46
G	44.33	1.90	42.45	3.05	104.42	0.42
H	60.14	1.83	52.76	3.73	113.98	1.70
I	bdl	NA	NA	NA	NA	NA
J	122.1	0.67	101.89	7.03	119.84	0.70
K	1683.6	3.08	1683.27	4.63	100.02	1.18
L	bdl	NA	NA	NA	NA	NA
M	85.14	4.00	86.22	21.3	98.75	0.98
N	20.96	3.12	18.55	12.64	112.97	0.52
O	bdl	NA	NA	NA	NA	NA
P	38.90	3.89	33.39	13.42	116.5	NA
			Pb			
A	136.03	8.63	119.60	64.13	113.74	3.20
B	3729	8.23	3070.11	30.44	121.47	1.20
C	1072	1.22	996.25	1.64	107.64	0.62
D	3790	0.47	3292.06	4.55	115.12	0.40
E	4121	4.04	4075.75	16.36	101.11	2.50
F	4.08	6.44	3.08	4.54	132.53	2.00
G	4.35	3.56	3.81	15.89	114.16	0.78
H	bdl	NA	NA	NA	NA	NA
I	bdl	NA	NA	NA	NA	NA
J	275.84	0.49	245.35	3.9	112.43	0.22
K	514.92	1.66	509.54	3.47	101.06	0.76
L	bdl	NA	NA	NA	NA	NA
M	70.73	0.24	66.29	17.21	106.69	0.08

N	62.25	7.02	58.53	6.55	106.36	2.32
O	3.25	13.07	3.34	61.88	97.38	2.78
P	383.07	3.83	343.08	10.13	111.66	1.66
			Se			
A	3.09	9.06	6.96	12.43	44.34	1.90
B	30.31	1.47	31.03	14.15	97.68	0.44
C	bdl	NA	NA	NA	NA	NA
D	34.7	4.01	28.40	4.77	122.17	1.22
E	26.73	1.37	25.90	11.05	103.2	0.40
F	7.10	6.93	1.4	83.34	507.16	1.66
G	5.54	14.00	1.73	47.46	320.11	3.22
H	bdl	NA	NA	NA	NA	NA
I	bdl	NA	NA	NA	NA	NA
J	5.14	8.53	3.20	42.1	160.53	1.94
K	245.39	3.15	257.17	2.89	95.42	1.26
L	bdl	NA	NA	NA	NA	NA
M	9.16	10.35	9.47	8.57	96.70	2.56
N	bdl	NA	NA	NA	NA	NA
O	bdl	NA	NA	NA	NA	NA
P	bdl	NA	NA	NA	NA	NA

BDL = Below detection limit.
NA = Not applicable.

Table 8. Method precision for Ca, Cu, Fe, Mg, Mn, and Zn with revised HorRat.

	Calcium			Copper			Iron			Magn	
Material	Mean, % (n=3)	RSD, %	Horrat(r)	Mean, mg/kg (n=3)	RSD, %	Horrat(r)	Mean, % (n=3)	RSD, %	Horrat(r)	Mean, % (n=3)	RS
A	0.869	1.07	0.52	6636.7	0.58	0.76	53.76	4.49	0.48	0.48	0
B	2.497	0.93	0.54	9123.2	1.33	1.98	24.95	2.58	2.1	2.204	0
C	0.51	1.94	0.88	11547	1.75	1.64	4.458	0.28	0.46	0.686	0
D	3.411	1.42	0.86	14284	1.88	1.6	13.96	3.67	2.74	3.928	1
E	2.797	1.57	0.92	9767.8	0.95	0.58	16.59	2.88	2.2	3.456	0
F	0.913	0.96	0.48	5421.2	1.06	0.24	1.074	0.74	0.38	1.304	
G	1.418	0.57	0.3	684.31	0.52	0.16	0.241	0.45	0.36	0.754	0
H	0.378	0.42	0.18	589.86	8.32	1.56	1.417	0.56	0.3	2.758	0
I	0.149	2.81	1.06	498.88	1.98	0.38	0.62	0.33	0.32	2.088	0
J	2.169	1.36	0.76	368.99	1.85	1.88	3.805	1.52	0.94	3.314	1
K	4.58	2.35	1.48	370.35	0.7	0.66	2.268	1.13	0.64	2.7	1
L	0.109	4.13	1.48	1800	1.86	0.4	2.103	0.63	0.36	0.336	0
M	1.724	0.52	0.28	3192.9	1.04	0.84	4.045	0.89	0.56	1.136	1
N	3.976	1.29	0.8	4651.4	2.39	1.86	8.927	1.15	0.8	0.774	0
O	2.493	1.88	1.08	3214.1	3.12	0.88	0.23	2	1.6	0.416	0
P	6.511	0.39	0.26	1757.3	1.8	0.6	23.82	3.95	3.18	0.824	

Fertilizer Subgroup of the Agricultural Materials Community

Statement of Method Need and Support

Trace metals in Fertilizer

In 2002 the fertilizer community began holding annual meetings (Fertilizer Metals Forum) to discuss their needs pertaining to methods of analysis of trace metals in fertilizers. This need rose primarily from a regulatory impetus to establish limits for certain metals. Results of this work included guidance for setting metals limits in fertilizers that formed the basis for the current proposed guidance published in the AAPFCO annual publication (publication #69) as Statement of Uniform Interpretation and Policy No. 25 (SUIP #25) available from http://www.aapfco.org/rules.html.

The second result was a fully collaborated method (AOAC 2006.03). This method came about as the result of input from the community between 2002 and 2006. While the method was successfully collaborated, it was done quickly in response to an urgent nation-wide need.. Several states had regulations in place but no "official" method. Any existing methods for the metals (primarily environmental methods) were not validated for fertilizers as a matrix. Fertilizers present a very unique matrix; it was determined that existing methods did not give reliable results due to high concentrations of salts, spectral interferences and ionization effects not properly controlled. The 2006 method was an improvement on the methodology used in the environmental sector, but still needed additional refinement as it was not optimized for all elements and interference posed by high levels of Iron.

With the success of the model, the Metals Forum evolved into the Methods Forum in 2008 to address a wide array of methods needs of the fertilizer community. Over the years hundreds of hours have been spent by dozens of volunteers discussing and forming proposals to establish science/risk based limits as well as develop and validate methods of analysis to monitor those limits.

The community continued to work on the improving the metals method and eventually requested that a revised method be collaborated that addressed the concerns of the community. Guidance to the study director was prepared to address the concerns and meet the needs of the community. Below are the primary charges to the study director and method champion.

The method must –

- Use equipment and instruments commonly available in state fertilizer laboratories –
- Utilize ICP-OES for detection, not ICP-MS as it is rarely available to state fertilizer labs
- Have detection limits that encompass the levels established in SUIP #25, but not overly aggressive avoiding undue time, acid quality and expensive clean room procedures
- Not be burdensome as it relates to digestion equipment or cross contamination
- Extend the current method to also encompass nutritive metals for greater efficiency
- Include a simple acid mixture of nitric and hydrochloric acids, avoid perchloric acid
- Ensure the greatest possible scope of materials be incorporated to include as many fertilizer matrices as possible, realizing that some sacrifices in performance would be worth the expanded scope.

The community has kept in close contact with the study director during method development and validation through face-to-face annual meetings and by the use of email and conference calls. The community has consensus that all of the above expectations are met in the currently proposed method as documented in *JAOAC* 97, pp 700-711 and as submitted to the Fertilizer ERP.

AOAC RESEARCH INSTITUTE

AOAC *OFFICIAL METHODS OF ANALYSIS* (OMA)

OMAMAN-28/AOAC 2006.03

Study Director: Sharon Webb, University of Kentucky, Division of Regulatory Services, 103 Regulatory Services Bldg, Lexington , Kentucky 40546-0275

TECHNICAL EVALUATION CRITERIA Is the test kit method scientifically and technically sound?	
ER 1	Yes
ER 2	Yes
ER 3	No, The method states it is intended for fertilizers but; 1). Targets some metals and ignores others (e.g. Al, Hg) that can be toxic to plants 2). Has a bias toward metals extractable by acid whereas alkali extraction is ignored. It is known that some metals are more available in alkali environments. 3). If a method is to be used for determining metals in fertilizers it should consider the pH range of soils wherein most crops are grown (pH 5.5-6.5) and, although environmental tests for metals may include highly acidic soils, this is not the case for agricultural soils where pH ranges are normally maintained within a specific range and may likely include alkali soils above pH 8.0. in areas of low rainfall or where irrigation waters contain high salts 4). If the purpose is to limit plant availability then a leachable metals test would be more appropriate than a total metals test considering that metals must be released from the fertilizer into soil solution in order for plant uptake to occur and only certain forms of some metals are plant available 5). To include plant macro- and micronutrients such as Ca, Mg, Fe, etc. in this method for total metals could be deceptive resulting in label guarantees for these fertilizer nutrients. This total metals would not be indicative of plant availability and would be doing a great disservice to the end user. This has already happened in some states where a label warning is being construed as a nutrient guarantee. 6.) As there are numerous methods for metals analysis if we are going to advance a method it should therefore have some value or indication of its solubility from the fertilizer material and potential plant availability (solubility in soil solution and leachability). This is not what this method is meant to determine (its scientific purpose).
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	No, The carbon interference/background for wavelengths below 250nm is not sufficiently addressed.
ER 2	Yes
ER 3	No, Should have both alkali and acidic measurements Should list pH of extractants. May have complexation with other elements during wait time

	which may decrease the final reading.
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Are the conclusions statements valid based upon data presented?	
ER 1	Yes
ER 2	Yes
ER 3	No, Not suitable for nutrients/metals that are increased in availability under alkali conditions or ones that may complex with other fertilizer constituents during extractant wait time.
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	Yes
ER 2	Yes
ER 3	No, In addition to the items mentioned in #1 above, the purpose of a fertilizer test should be to determine the availability of any given metal/nutrient for plant uptake once applied to the soil. This method provides no such proof of it correlating in any way with plant uptake. Also, the

	availability of metals can be affected by their concentration and other ions present, among other things.
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
General Comments about the Method Scope/Applicability:	
ER 1	Sound methodology but it does not address the hardware (actual ICP differences) sufficiently. Without addressing this there will be biases based on plasma configuration used. Especially for the heavy metals.
ER 2	Authors are to be commended for undertaking this important correction and addition of additional elements to make this a more universal method for fertilizer analysis.
ER 3	The method states it is intended for fertilizers but; 1). Targets some metals and ignores others (e.g. Al, Hg) that can be toxic to plants 2). Has a bias toward metals extractable by acid whereas alkali extraction is ignored. It is known that some metals are more available in alkali environments. 3). If a method is to be used for determining metals in fertilizers it should consider the pH range of soils and crops, and, although an environmental test for metals may include highly acidic soils, this is not the case for agricultural soils where pH ranges are normally maintained within a specific range and may likely include alkali soils above pH 8.0. 4). If the purpose is to limit plant availability then a leachable metals test would be more appropriate than a total metals test considering the metals must be released from the fertilizer into soil solution in order for plant uptake to occur 5). To include plant nutrients such as Ca, Mg, Fe, etc. in this method for total metals could result in label guarantees for these fertilizer nutrients and an acid extraction for total metals would not be indicative of plant availability and would do a disservice to the end user. This has already happened in some states where a label warning is

	being construed as a nutrient guarantee. If we are going to advance a method it should therefore have some value or indication of its solubility from the fertilizer material and potential plant availability. This is not what this method is meant to determine.
ER 4	Looks good.
ER 5	I think this method is taking advantage of the technology that is available. The simultaneous determination of the metals on ICP will be very beneficial to laboratories.
ER 6	more studies have to be done in order to improve the recovery of some elements
ER 7	Scope and applicability for ferilizers is appropriate for the specified metals.
ER 8	Webb, S., Bartos, J., Boles, R., Hasty, E., Thuotte, E., & Thiex, N. J. (2014). Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry Detection: Single-Laboratory Validation of a Modification and Extension of AOAC 2006.03. Journal of AOAC International, 97(3), 700-711. The paper describes a single-laboratory validation study for the simultaneous determination of arsenic, cadmium, calcium, cobalt, copper, chromium, iron, lead, magnesium, manganese, molybdenum, nickel, selenium, and zinc in all major types of commercial fertilizer products by microwave digestion and inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis. The importance of the proposed method is correlated to the possible extension and modification of the current AOAC 2006.03 (determination of arsenic, cadmium, cobalt, chromium, lead, molybdenum, nickel and selenium, also named “Group A”, in fertilizers) with the inclusion of calcium, copper, iron, magnesium, manganese, and zinc (also named “Group B”). The use of a dual acid digestion system - hydrochloric and nitric acids – instead of simple nitric acid is proposed as modification. On the basis of obtained results, the proposed method is reported to: a) Assure a significant increase in laboratory efficiency when compared to the use of both AOAC Methods 965.09 – Nutrients (Minor) in Fertilizers, Atomic Absorption, Spectrophotometric Method - and 2006.03. AOAC 695.09 is considered because of the necessity of validating results for calcium, copper, iron, magnesium, manganese, and zinc b) Assure a more efficient recovery of several metals in comparison with AOAC 2006.03 c) Meet the criteria recommended by the Association of American Plant Food Control Officials (AAPFCO) Laboratory Services Committee. The applicability of the proposed method has been declared. It has to be noted that AOAC 2006.03 mentions the use of nitric acid, while the proposed technique uses also hydrochloridric acid (in both situations, fertilizers can produced exothermic reactions) in a closed vessel microwave digestion system at 200 °C. The use of microwave digestion units has to be carefully considered.

	17 materials have been utilized for the validation, including two samples for the determination of accuracy; in addition, data demonstrating calibration and linearity, accuracy, precision, comparability, working range, LOD/LOQ estimation, and ruggedness tests are present. In general, the document method performance is compliant with Standard Method Performance Requirements (SMPR) criteria.
ER 9	Method is well written and data presented supports most conclusions. Lot's of good work done in the study and background research. well thought out and much needed.
ER 10	None
Pros/Strengths of the Manuscript:	
ER 1	QC and analytical methodology is fine
ER 2	The purpose of the study is easy to understand and the data represents marked improvement over AOAC 2006.03 for Group B metals. The ruggedness testing section is excellent and well-done.
ER 3	Will expedite lab analyses and have cost savings
ER 4	Well written and validated
ER 5	The manuscript references many different methods, used Magruder samples for consistency.
ER 6	fast analysis
ER 7	Clear explanation of the results
ER 8	The paper considers the following aspects with high attention: 1) Choice of samples for validation. 15 of the 30 original collaborative study materials from 2006.03 have been used for method validation. In addition, NIST SRM 695 and Magruder 2009-06 have been incorporated as validation materials 2) Description of needed equipments and reagents 3) Description of operative procedures, from the description of the principle of the proposed method and instrumentation to calculations and quality control tests 4) Validation study: Calibration and Linearity; Trueness or Accuracy; Precision; Comparability; Working range; LOD and LOQ estimation; Ruggedness tests. In detail, Authors claim (preliminary study results – Appendix - comparison of the new method with AOAC 965.09, AOAC 2006.03, hot block-acid digestion and microwave acid digestion) that the simultaneous digestion with two acids and the subsequent ICP-OES analysis allow a notable recovery for both metal Groups. There are not significant differences between AOAC 965.09 and the proposed method with relation to Ca, Cu, Mn and Zn (P = 0.05), while differences are significant for Mg (P level of 0.05). The same situation is observed when comparing the proposed extension and AOAC 2006.03. For this reason, it may be assumed that Mg should be recovered with addition of HCl. The same situation is observed with iron; in addition: a) Should Fe be derived from organic materials, AOAC 965.09 Part C method would not be applicable b) Should Fe be in the range: 10-50 %, the mixed digestion method should demonstrate slightly

different results if compared with AOAC 965.09
c) AOAC 2006.03 does not adequately recover Fe if compared with other methods, including AOAC 965.09.
As a consequence, it appears that the addition of HCl to the digestion system is useful.

With relation to Group A- metals, comparisons have been made with AOAC 2006.03 and the hot-block system. In general, there are not statistical differences (P = 0.05) except for Co and Mo; in addition, Cr shows high statistical differences (P = 0.01): this behaviour can be interpreted as a possible bias of AOAC 2006.03.

The following strengths of the proposed method have to be also mentioned:

Calibration and linearity
The most suitable type of calibration curve has been determined (linear or quadratic) using the software provided by the instrument. For this reason, the calibration should be easy enough.
Observed correlation coefficient values are between 0.99909 and 1.000000.
Linearity has been determined by comparing responses for the working standard solutions over the range of expected concentrations. All results reported have undergone corrective calculations to include the test portion weight and total volume to yield the corrected values.

Trueness or Accuracy
Accuracy has been determined by results of analysis for all elements of interest, Group A and Group B, in NIST SRM 695 Trace Elements in Multi-Nutrient Fertilizer. Additionally, the results by the proposed method have been compared to consensus values for Magruder 2009-06, and expressed as percentage recovery of the certified or consensus value.
Generally, percent recovery results are in the range: 74.6 – 102.5 (for NIST SRM 695) and 94.9-149 (for Magruder 2009-06).

Precision
Method precision has been determined by independent analysis of the validation materials in triplicate, and variation has been expressed as relative standard deviation (RSD). Many results show HorRat values below 0.3 for a single validation study. In detail, HorRat values (total samples: 15) are below 0.3:
a) seven times for calcium
b) five times for cuprum
c) eight times for iron (in addition, two HorRat values are reported are reported to be > 1.3)
d) 12 times for magnesium
e) three times for manganese (in addition, five HorRat values are reported to be > 1.3)
f) three times for zinc (in addition, two HorRat values are reported to be > 1.3).

However, RSD values for the proposed method are low if compared with collaborative study

	2006.03 results. Two examples can be made with concern to cobalt and nickel (Group A). a) Cobalt. Material 'P', result: 0.52 vs 0.57 (collaborative study), RSD = 20.46 vs 66.74 (collaborative study) b) Nickel. Material 'P', result: 38.90 vs 33.39 (collaborative study), RSD = 3.89 vs 13.42 (collaborative study). In general, RSD for Group B metals remain low enough. Comparability Comparability to AOAC 2006.03 for Group A elements has been calculated by testing 15 of the original collaborative study materials with both digestion methods. Data are expressed as a percentage recovery of the original grand average result. Many results show higher recovery values. Probably, these results demonstrate the enhanced recovery "power" of the proposed method, on the basis of RDS values. Working Range The working range of the method is determined by the working calibration standards. Each laboratory should determine the range best suited to their instrument's capability. In general, 10 % extrapolation from the highest calibration standard often produces acceptable results for Group A and B elements. LOD and LOQ With reference to Group A, the paper shows the instrument LOD (estimated with standard solutions) and the method LOQ and LOD (estimated with validation materials). Interestingly, the LOQ for Group B elements are completely dependent upon the calibration range since the method is not working close to the instrument limits. Ruggedness trial Generally, results have not shown appreciable effects from the deviations of the method. Obtained results (differences) are all within normal variation and indicate that the method is sufficiently rugged with respect to the conditions studied.
ER 9	Significant work was done and data generated; information presented well. Method presents a step forward in speed, safety and scope. Digestion is relatively simple and straightforward, with few chances for human error beyond sample weight. Updating the instrumentation to ICP-OES is very important. Scope and ranges are well covered by the materials used. Digestion options are well researched.
ER 10	Robust Method
Cons/Weaknesses of the Manuscript:	
ER 1	Some of the optimization steps are dated and based on studies from 30 years ago. System hardware and performance has changed and this needs to be considered. I.E. Meremt's robustness test is a guideline for plasma conditions BUT NOT for analytical optimization of the method/ICP. Also, Cs is a buffer for consideration on Axial/DV/TI systems, it is not typically used

	in radial ICP systems. These need to be addressed because they can have an adverse affect based on system hardware used.
ER 2	Perhaps more emphasis could be placed on Section F (e) regarding interelement interference in instruments that don't have software to correct for this issue.
ER 3	Favors metals that are extractable in acid rather than alkali Does not provide extractant pH levels Reports total metals which is not indicative of plant availability Could tend to mislead consumers if construed as a micronutrient label guarantee Not an appropriate method for fertilizer materials in determining plant availability
ER 4	N/A
ER 5	"cut" (page 112) could be replaced.
ER 6	may not be applicable foe all fertilizer products
ER 7	none
ER 8	The following weaknesses of the proposed method may be mentioned: Calibration and linearity Some deconvolution effects have to be considered (Fe, Co) when speaking of certain wavelengths for arsenic, cadmium, lead and selenium (calibration range: 0-10 µg/mL). Precision Many results show many HorRat values below 0.3 for a single validation study. However, RSD values for the proposed method are extremely reduced if compared with collaborative study 2006.03 results. In addition, RSD for Group B metals remain low enough, in general. LOD and LOQ It should be noted that lower limits for Group B elements can be achieved by altering the test portion size or choosing more sensitive wavelengths. Ruggedness trial It has to be noted that: 1) Se values in the sample are at or below the method LOQ. For this reason, the proposed method is not recommended for Se 2) Certain 'Relative percent difference' values show that the determination of iron has to be carried out carefully. This element is reported to be the most variable element and ruggedness test results seem to confirm the affirmation.
ER 9	All of the ruggedness work was done on the digestions portion of the study, but none was done on ICP variables. How software varies from instrument to instrument may yield different lines

	used and de-convolution techniques. Additional work needs to be done here to better guide future users.
ER 10	Little discussion of what materials may not be suitable for analysis by this method.
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	Partially, again without knowing the ICP plasma configuration used by each participating lab, there maybe biases in the results based on the plasma orientation used with regards to the recommended methodology (ie. Internal Standards recommended).
ER 2	Yes, for a modification an extension of AOAC 2006.03.
ER 3	YES
ER 4	Yes
ER 5	This was a single laboratory study.
ER 6	n/a
ER 7	yes
ER 8	The current manuscript is a single-laboratory validation study. The available data support the method as written.
ER 9	Yes, however data on a few more materials may be needed to support the full scope of "fertilizers". ICP variables may need to be researched (ruggedness) to determine where variation can be tolerated based on instrument differences.
ER 10	Yes, appears to.
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	Yes
ER 2	Yes, dramatic improvement in RSD% are evident.
ER 3	YES
ER 4	Yes
ER 5	Se values in the sample are at or below the method LOQ (Pg 109)
ER 6	n/a
ER 7	yes
ER 8	The current manuscript is a single-laboratory validation study.
ER 9	I did not see a collaborative study protocol beyond the information provided in the JAOAC article. If there is a protocol available please provide a link to it or the file itself.
ER 10	Yes, appears to.
Are there any concerns regarding the safety of the method?	
ER 1	No concerns
ER 2	No
ER 3	Yes, what about Na and NO3 potential reactions with other constituents?
ER 4	No
ER 5	Yes, Safety Advisor has addressed.
ER 6	no
ER 7	No

ER 8	At present, there are no safety concerns with the exception of the contemporary use of nitric and hydrochloridric acids. In addition, the use of microwave digestion units at 200 °C has to be carefully considered.
ER 9	I have not seen a review by the safety advisor. The safety checklist seems appropriate. I do not have any additional concerns regarding the safety of this method.
ER 10	No cautions provided
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	No concerns
ER 2	No
ER 3	unknown, no access to this
ER 4	No
ER 5	none
ER 6	no
ER 7	No
ER 8	At present, there are no concerns with relation to data manipulation, data tables and statistical analysis.
ER 9	I have not seen a review by the statistical advisor. However I do not have any concerns regarding the statistics used to support this method other than the use of a broader selection of materials (organic derivation) used in future work or data reviews.
ER 10	None observed.
EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes

ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Could some be omitted and covered by a simple statement?	
ER 1	No
ER 2	No
ER 3	Yes, Table 1.Could be a summation of the number and types of fertilizer materials used.
ER 4	No
ER 5	No
ER 6	No
ER 7	No
ER 8	No
ER 9	No
ER 10	No
Are the references complete and correctly annotated?	

OMAMAN-28/AOAC 2006.03

Study Director: Sharon Webb, University of Kentucky, Division of Regulatory Services, 103 Regulatory Services Bldg, Lexington , Kentucky 40546-0275

ER 1	No, Outlier ratio should be described (ie. 0/0 versus 1/0)
ER 2	Yes
ER 3	No, I would think the Magruder samples and testing which are used quite frequently within the text should be listed as a reference. Shouldn't method 965.09 also be referenced similarly to 2006.03?
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes
Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
ER 8	Yes
ER 9	Yes
ER 10	Yes

SPECIAL GUEST EDITOR SECTION

Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry Detection: Single-Laboratory Validation of a Modification and Extension of AOAC 2006.03

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A single-laboratory validation study was conducted for the simultaneous determination of arsenic, cadmium, calcium, cobalt, copper, chromium, iron, lead, magnesium, manganese, molybdenum, nickel, selenium, and zinc in all major types of commercial fertilizer products by microwave digestion and inductively coupled plasma-optical emission spectroscopy analysis. This validation study proposes an extension and modification of AOAC 2006.03. The extension is the inclusion of calcium, copper, iron, magnesium, manganese, and zinc, and the modification is incorporation of hydrochloric acid in the digestion system. This dual acid digestion utilizes both hydrochloric and nitric acids in a 3 to 9 mL volume ratio/100 mL. In addition to 15 of the 30 original validation materials used in the 2006.03 collaborative study, National Institute of Standards and Technology Standard Reference Material 695 and Magruder 2009-06 were incorporated as accuracy materials. The main benefits of this proposed method are a significant increase in laboratory efficiency when compared to the use of both AOAC Methods 965.09 and 2006.03 to achieve the same objective and an enhanced recovery of several metals.

Kane et. al. (1, 2) developed a standard analytical method, AOAC 2006.03, for the determination of the metals, arsenic (As), cadmium (Cd), cobalt (Co), chromium (Cr), lead (Pb), molybdenum (Mo), nickel (Ni), and selenium (Se) in fertilizers as an answer to the Association of American Plant Food Control Officials (AAPFCO) call for a Statement of Uniform Interpretation and Policy (SUIP No. 25; 3). This policy addresses the acceptable levels of specific metals in fertilizers. Utilized together, the analytical method and SUIP allow commercial fertilizers to be monitored for metal levels so that informed decisions can be made to ensure that the longterm quality of soil and food is protected. This investigation led to the formation of the Metals Forum in 2002, and evolved into the Methods Forum, which is comprised of chemists from regulatory, academia, industry, commercial, and private laboratories as well as instrument vendors. This forum meets annually to discuss how to best satisfy the rising call for new or improved fertilizer methods; decreased turnaround time; new instrument technology and efficiencies; QC; and new state, federal, or trade regulations and to address method needs as new commercial fertilizer products are introduced.

This proposed method modification and extension addresses several of the aforementioned needs. By combining everything into one single digestion and detection technique, substantial efficiencies are realized. This method will simultaneously determine As, Cd, calcium (Ca), Cr, Co, copper (Cu), iron (Fe),

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An appendix is available on the *J. AOAC Int.* website, http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac

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Table 1. Validation materials used for the microwave mixed acid SLV study

SLV ID	ID from 2006.03 Collaborative Study	Description
A	4321/2025	Metal Fe oxysulfate
B	4031/5938	Magruder 2002-09B
C	5488/5890	Zinc oxysulfate
D	2818/7669	Granulated mine waste
E	1615/2056	Metal oxysulfate
F	3313/6267	Western MAP[a]
G	7999/3375	DAP from North African rock
H	6501/4812	NC MAP
I	7738/7418	China DAP[b]
J	3917/8165	Magruder 2003-11
K	4459/8931	Magruder 2004-07
L	8873/9469	N-P-K lawn product blend
M	9886/9774	Organic biosolid
N	4626/8088	Organic mixed fertilizer + biosolid
O	6411/3401	Composted manure
P	3716/4606	Fe humate
Q	NA[c]	NIST SRM[d] 695
R	NA	Magruder 2009-06

[a] MAP = Monoammonium phosphate.

[b] DAP = Diammonium phosphate.

[c] NA = Not applicable.

[d] NIST SRM = National Institute of Standards and Technology Standard Reference Material.

Pb, magnesium (Mg), manganese (Mn), Mo, Ni, Se, and zinc (Zn) in all types of fertilizers. For the purpose of this paper, the metals in the **2006.03** paper (1) will be referred to as "Group A metals" (As, Cd, Cr, Co, Pb, Mo, Ni, and Se). Those proposed elements to be added to the scope will be referred to as "Group B metals" (Ca, Cu, Fe, Mg, Mn, and Zn).

Background

In 2008, a small interlaboratory study involving four laboratories was undertaken by a group of regulatory and industry chemists, led by the Office of the Indiana State Chemist (4). Based upon the results of the interlaboratory study, a single-laboratory validation (SLV) study was initiated to determine if a universal method could be viable for both Group A and Group B metals in all classes of fertilizers and to evaluate if using mixed acids rather than a single acid enhances recoveries. A complete description of the initial study is found in an Appendix on the *J. AOAC Int.* website (http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac).

Methods and Materials

Validation Materials

Fifteen of the 30 original collaborative study materials from **2006.03** were used for method validation. In addition, NIST SRM 695 and Magruder 2009-06 were incorporated as validation materials. Magruder 2009-06 was included because it was formulated to contain measurable amounts of all the Group A metals and because consensus values were available from the Magruder Check Sample Program. NIST 695 was included because of the availability of either a certified or reference value for all of the elements for which the method was validated (*see* Table 1).

The proposed modification and extension of AOAC *Official Method*℠ **2006.03** is described below.

Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and ICP-OES Detection

Scope

The method is applicable to the determination of As, Cd, Co, Cr, Pb, Mo, Ni, and Se and to the determination of Ca, Cu, Fe, Mg, Mn, and Zn in all classes of fertilizers.

Safety

Observe standard precautions when handling concentrated acids and acid digests. When dispensing acid or venting vessels, use gloves, eye and face protection, and a laboratory coat. Never remove hot vessels from the microwave; wait until they are near room temperature. Keep microwave door closed while vessels are hot. The door is the primary safety device if a vessel vents.

Proposed Digestion Method

A. Principle

Test portion is heated with either nitric acid (option 1) or with nitric and hydrochloric acids (option 2) in a closed vessel microwave digestion system at 200°C.

B. Apparatus

Microwave.—Commercial microwave designed for laboratory use at 200°C, with closed vessel system and controlled temperature ramping capability. It is recommended that a vessel design be selected that will withstand the maximum possible pressure, since some organic fertilizer products, and also carbonates if not given sufficient time to predigest, will generate significant pressure during digestion. (Vessels can reach 700 psi or more on occasion.) Vent according to manufacturer's recommendation. (*Caution*: Microwave operation involves hot pressurized acid solutions. Use appropriate face protection and laboratory clothing.)

C. Reagents (Option 1 Applicable to Group A Metals Only): Nitric Acid Digestion

(a) *Water.*—Use 18 Megaohm water throughout.

(b) *Concentrated HNO_3.*—Use trace metal grade HNO_3

throughout (nitric acid–HNO_3, 67–70%, OmniTrace grade; EMD Chemicals, Darmstadt Germany).

D. Determination

(a) *Option 1 (applicable to Group A metal only).*—Refer to *Official Methods of Analysis, Official Method*℠ **2006.03D.**

(b) *Option 2 (applicable to Group A and Group B metals).*—Prepare solid materials according to AOAC Method **929.02.** Accurately weigh 1.000 ± 0.10 g (0.500 g for organic matrixes) test portion and transfer to digestion vessel. Use a weighing paper insert to line the vessel walls during transfer, which will keep test portion from adhering to the sides of vessel. Fluid materials may be weighed directly after mixing. Add 9.0 ± 0.2 mL trace metal grade HNO_3, **G(b),** allow samples to sit for approximately 20 min, and then add 3.0 ± 0.2 mL HCl, **G(c).** Loosely cap vessels without sealing, predigest at room temperature until vigorous foaming subsides, or overnight if time allows. Seal vessels according to manufacturer's directions and place in microwave. With power setting appropriate to microwave model and number of vessels used, ramp temperature from ambient to 200°C in 15 min. Hold at 200°C for 20 min. Cool vessels according to manufacturer's directions, vent, and transfer digests to 100 mL volumetric flasks. Rinse each digestion vessel three times with approximately 10 mL water, **C(a),** and transfer rinse solution to the volumetric flask; dilute flask to volume with water, **C(a),** and mix. Filtering the digestate is optional, but necessary if problems with nebulizer clogging are experienced. Transfer to polypropylene, or other suitable, containers within 2 h, unless solutions are to be analyzed immediately. Dilute any digestates that are found to be above the standard curve range. Secondary dilutions require addition of appropriate amounts of HNO_3 and HCl to maintain the proportion of 9% HNO_3 and 3% HCl in the final solution to be analyzed.

Proposed Detection Method

E. Principle

Digested test solution, or an appropriate dilution, is presented to the inductively coupled plasma-optical emission spectrometry (ICP-OES) instrument calibrated with acid matched standard calibrant solutions. An ionization buffer (cesium) is used to minimize easily ionized element (EIE) effects, and scandium and/or beryllium are used as internal standard(s).

F. Instrumentation and Configuration

(a) *ICP optical emission spectrometer.*—Capable of determining multiple wavelengths for each element of interest. A 3-channel peristaltic pump is desirable to avoid the necessity of having to manually add ionization buffer and internal standard to each test solution. Use a Meinhard or Seaspray nebulizer and Cyclonic spray chamber, or other components designed to optimize aerosol formation and maximize precision. Select sample and internal standard pump tubes, and peristaltic pump rotation speed, with regard to manufacturer's recommendations, but try to keep sample and internal standard pump tubes of similar size, to maximize mixing accuracy, while maintaining needed detection levels.

The analyst must compensate for EIE effects in the plasma since fertilizer materials can contain substantial concentrations of elements that provide a significant source of electrons to the plasma, such as K and Ca. The presence of an ionization buffer in all test solutions and standards will minimize the effect of varying concentrations of EIEs in the sample. Power settings and nebulizer gas flow should be optimized for robust plasma conditions. The analyst needs to ensure that the Mg 285.213:Mg 280.271 ratio (Mermet principle of robust plasma) demonstrates robust operating conditions in accordance with the ratio established by the instrument manufacturer. Two to three replicate readings with relatively long integration times are recommended to improve precision and detection capabilities. Properly matched test solution and calibration matrices and optimized instrument settings should result in internal standard ratios for most test solutions consistently in the range of 0.9 to 1.1. It is not typical to have the ratio lower than 0.8 over a very wide range of fertilizer material types. The occurrence of lower ratios is cause for troubleshooting. Select ionization buffer/internal standard solution, G(f), such that after mixing unknown and internal standard solutions using the instrument's peristaltic pump, the combined solution presented to the nebulizer contains ≥2200 mg/kg cesium chloride; 0.75 to 1.0 mg/kg internal standard; and ≤7.2 mg/mL actual fertilizer material. [For example, these conditions would be met with a 1 g test portion digested and diluted to 100 mL; an ionization buffer/internal standard solution of 8000 mg/kg cesium chloride and 3 mg/kg scandium and/or beryllium internal standard(s); and pump tubes of white/white (1.02 mm id) sample and orange/white (0.64 mm id) internal standard, the white/white contributing about 72%, and the orange/white contributing about 28%, to the final nebulized solution.] All analytical wavelengths should be corrected using an internal standard wavelength.

However, best practice is to utilize similar transitions between analyte and internal standard. For example, the 188.980 wavelength is from arsenic in the atomic state, so the internal standard wavelength used for correction should also be from the atomic state, such as Sc 361.383. Conversely, match ionic sample lines with ionic internal standard lines. (*Note*: Do not use yttrium

Table 2. Recommended inductively coupled plasma-optical emission spectrometry wavelengths for Group A and B metals

Element	Wavelength(s)
As	188.980[a], 193.696[a]
Ca	183.944, 318.127, 430.253
Cd	214.439[a], 226.502[a], 228.802
Co	228.615, 230.786, 258.033
Cr	205.560, 267.716, 276.653
Cu	217.895, 222.778, 324.754, 327.395
Fe	234.350, 238.204, 240.489, 259.837
Mg	277.983, 278.297, 285.213, 383.829
Mn	260.568, 261.815, 263.817, 293.931
Mo	202.032, 204.598
Ni	216.55[a], 222.486, 231.604
Pb	220.35[a]
Se	96.02[a]
Zn	206.200, 213.857, 334.502, 472.215

[a] Wavelengths with potential spectral interference.

Table 3. Preparation of working standards

Std	Final working std concn, mg/L	Intermediate Std [G(l)], mL	Stock Std [G(m)], mL		HNO_3[a] [G(b)], mL	HCl[a] [G(c)], mL	Final vol., mL
Blank	0	0	0	0	90	30	1000
1	0.1 A/0.5 B	5			45	15	500
2	0.5 A/1 B	25			45	15	500
3	1 A/3 B	50			90	30	1000
4	5 A & B		5		90	30	1000
5	10 A & B		5		45	15	500
6	30 B			3	90	30	1000
7	50 B			5	90	30	1000
8	80 B			4	45	15	500
9	100 B			10	90	30	1000
10	200 B			10	45	15	500
11	300 B			15	45	15	500
12	400 B			20	40	15	500
13	600 B			30	40	15	500
14	10 A		5		45	15	

[a] Since commercial stock standards are often stored or preserved in acids, the volume of HNO_3 and HCl added to the calibration standards should be adjusted to include any contribution of HNO_3 and/or HCl from the commercial stock standard source.

as an internal standard, since it is found native at low levels in some phosphate ore sources.)

(b) *ICP wavelengths.*—A number of wavelengths may be used for analysis of the elements of interest, depending on the capability of the analytical instrument used. At a minimum, select at least two wavelengths for each element of interest and report the averaged value of closely agreeing results, with the exception of lead and selenium, for which there is only one reliable wavelength available. Table 1 provides a list of suggested wavelengths, not in any order of preference, that have been found acceptable for most fertilizer materials. Other lines of appropriate sensitivity free of interferences or corrected for interferences may be just as acceptable. However, it is imperative that instrument response (both the wavelength peak scan and the calculated concentration) be reviewed for each test solution and element. Fertilizer materials are extremely variable in composition, and a wide concentration range of potential interfering elements is expected, so no single wavelength will work in every instance. Occasionally, data with an interference will inevitably be found and must be eliminated from inclusion in the mean calculation result for that particular element and sample.

(c) *Wavelength interference treatment.*—Interelement interference can cause substantial error in analytical result. Error can be minimized by several techniques: (*1*) Three or more analytical lines may be used for a given element, and when an interferent is present in a particular line, the result for that line is omitted from the mean value reported. (*2*) Some ICP software has the capability of mathematically modeling potential interferents and deconvoluting the instrument response into an analytical element portion and an interferent portion. (*3*) Interelement correction is an alternative mathematical technique to use with instruments for which mathematic modeling is not available, or where direct spectral overlap negates use of the deconvolution technique. The following lines, if used, must utilize one of the correction techniques; corrections for other wavelengths may be applied as needed and appropriate: (*1*) As 188.980: Correct for Cr interference, or verify that Cr is not present at an interfering level in the test portion analyzed. (*2*) As 193.696: Fe affects the arsenic peak. Remove with an Fe model, or verify that Fe is not present at an interfering level in the test portion analyzed. (*3*) Cd 214.439 and 226.502: Fe, present in many fertilizers, interferes with both suggested Cd wavelengths. Mathematically correct instrument Cd response for the interference, or verify analytically that Fe is not present at an interfering level in the test portion analyzed. (*4*) Pb 220.353: Mathematically correct instrument Pb response for Fe interference, or verify that Fe is not present at an interfering level in the test portion analyzed. (*5*) Se 196.026: Mathematically correct instrument Se response for Fe interference, or verify that Fe is not present at an interfering level in the test portion analyzed.

(d) *ICP instrument calibration.*—Prepare Group A working standard solutions from 1000 mg/L commercial stock standards. Custom blended multielement stock standard in an acid ratio (9% HNO_3:3% HCl) is acceptable. Working standards should be prepared at concentrations listed in Table 3, if they fit the sensitivity of the available instrumentation. Calibration concentrations should be adjusted to match the sensitivity of an instrument. However, linear curves should have correlation coefficients of at least 0.999 and a standard error of no more than 10%. Quadratic calibrations should have a correlation coefficient of at least 0.999, a standard error of less than 10%, a curvature of no more than 25%, and an upward curvature of no more than 400%.

G. Reagents (Option 2): Dual Acid Digestion

(a) *Water.*—Use 18 Megaohm water.

(b) *HNO_3.*—Use trace metal grade HNO_3 (nitric acid–HNO_3, 67–70%, OmniTrace grade; EMD Chemicals).

Table 4. Optimization factors for ruggedness testing

No.	Optimization factor	Major value	Minor value
1	Acid ratio (HNO_3:HCl)	A = 10:2	a = 8:4
2	Test portion, g	B = 1.200	b = 0.8000
3	Digestion time, min	C = 25	c = 15
4	Digestion temp., °C	D = 220	d = 180
5	Dilution vol., mL	E = 110	e = 90
6	Filtering	No	Yes
7	Dilution	Weight	Volume

(c) *HCl.*—Use trace metal grade HCl (hydrochloric acid–HCl, 35–38%, trace metal grade; Cat. No. A508-500, Fisher Scientific, Pittsburgh, PA).

(d) *Triton X-100 solution.*—Triton X-100.—Octyl phenol ethoxylate (J.T. Baker Chemicals, Center Valley, PA).

(e) *0.5% Triton X-100 solution.*—Dilute 0.5 mL Triton X-100 G(d), to 100 mL with H_2O, G(a).

(f) *Cesium chloride.*—Formula weight 168.36, trace metal basis, purity >99.999%, Cat. No. 203025-50G (Sigma-Aldrich, St. Louis, MO).

(g) *1000 mg/L Sc standard.*—In 4% HNO_3, Product No. 100048-1 (High Purity Standards, Charleston, SC).

(h) *1000 mg/L Be standard.*—In 4% HNO_3, Product No. 1005-1 (High Purity Standards).

(i) *Ionization buffer/internal standard solution.*—Weigh 8.0 g CsCl, G(f), into a 1000 mL acid-washed volumetric flask. Add 3 mL each of ICP grade scandium, G(g), and beryllium, G(h), 1000 mg/L stock solution, as internal standards. Also add 1 mL of 0.5% Triton X-100, G(e), dilute to volume, and mix. Store in a polypropylene bottle. (*Note*: Reagent concentrations assume the use of white/white, 1.02 mm id sample pump tube, and orange/white, 0.64 mm id internal standard pump tube. If the test solutions and internal standard solutions are mixed in different proportions by the instrument's peristaltic pump, then adjust the reagent concentrations to meet concentration requirements of mixed solution nebulized by the instrument, as outlined in F. Note that sample and internal standard solution mixing ratio is proportional to pump tube flow rates, not proportional to pump tube IDs.)

(j) *Stock standard solutions, As, Cd, Co, Cr; Pb, Mo, Ni, and Se.*—Working standards can be prepared from ICP grade 1000 mg/L commercial stock standard solutions for As, Cd, Co, Cr, Pb, Mo, Ni, and Se. A number of companies provide this stock standard service.

(k) *Stock standard solutions, Ca, Cu, Fe, Mg, Mn, and Zn.*—Working standards can be prepared from 10 000 mg/L individual element ICP grade commercial stock standard solutions for Ca, Cu, Fe, Mg, Mn, and Zn. However, it is also acceptable to use commercially prepared custom blended stock standard mixtures containing some or all elements at stock concentrations. A number of companies provide this stock standard service.

(l) *10 mg/L intermediate standard solution for preparation of low-level working standards for As, Cd, Co, Cr; Pb, Mo, Ni, and Se.*—Dilute 5.0 mL of stock 1000 mg/L standard solution, G(j), to 500 mL. Prepare fresh each time standards are prepared, and use immediately after preparation.

(m) *50 mg/L intermediate standard solution for preparation of low-level working standards for Ca, Cu, Fe, Mg, Mn, and Zn.*—Dilute 5.0 mL of stock 10 000 mg/L standard solution to 1000 mL. Prepare fresh each time standards are prepared, and use immediately after preparation.

(n) *Working standard solutions (see Table 3) for Ca, Cu, Fe, Mg, Mn, and Zn.*—Standards should have the same acid concentration as digested test solutions. Date all calibration solutions when made, which are stable at room temperature for 60 days. Monitor standard curve fit and intensity for signs of change and degradation over time. (*Note*: Based on instrumentation, the calibration standards may be adjusted to fit the manufacturer guidelines regarding standard curve requirements. However, linear curves should have correlation coefficients of at least 0.999 and a standard error of no more than 10%. Quadratic calibrations should have a correlation coefficient of at least 0.999, a standard error of less than 10%, a curvature of no more than 25%, and an upward curvature of no more than 400%.)

(*1*) *10 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 100.0 mL intermediate standard solution, G(m), into a 500 mL acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to acid-washed polypropylene bottle.

(*2*) *5 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 100 mL of combined 50 mg/L element stock solution into a 1000 mL acid-washed volumetric flask. Add 90 mL trace metal grade HNO_3 and 30 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*3*) *1 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 50.0 mL of 10 mg/L intermediate stock solution into a 500 mL acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*4*) *0.5 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 25.0 mL of 10 mg/L intermediate stock solution into a 500 mL acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*5*) *0.1 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 5.0 mL of 10 mg/kg intermediate stock solution into a 500 mL acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*6*) *600 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 30.0 mL of single element or combined 10 000 mg/L multielement stock standard solution into a 500 mL acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to acid-washed polypropylene bottle.

(*7*) *400 mg/kg Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 20.0 mL of single element or combined 10 000 mg/L multielement stock solution into a 1 L acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*8*) *300 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 15.0 mL of single element or combined 10 000 mg/L multielement stock standard solution into a 500 mL acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*9*) *200 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 10.0 mL of single element or combined 10 000 mg/L multielement stock

Table 5. Calibration wavelength, relative intensity, curve type, range, correlation coefficient, and error

Element	Wavelength, nm	Relative intensity	Curve	Deconvolution	Calibration range, µg/mL	Observed correlation coefficient	Error[a]
As	188.98	Weak	Linear	Fe	0–10	0.99993	10
As	193.696	Weak	Linear	Fe	0–10	0.99999	10
Ca	183.944	Intermediate	Quadratic	None	0–600	0.999448	10[a]
Ca	318.127	Intermediate	Quadratic	None	0–600	0.999687	10[a]
Ca	430.253	Intermediate	Quadratic	None	10–600	0.999711	10[a]
Cd	214.439	Strong	Linear	Fe	0–10	0.999243	10
Cd	226.502	Strong	Linear	Fe	0–10	0.999642	10
Cd	228.802	Strong	Linear	None	0–10	0.999996	10
Co	228.615	Strong	Linear	None	0–10	0.999957	10
Co	230.786	Strong	Linear	None	0–10	0.999997	10
Co	258.033	Strong	Linear	None	0–10	0.999998	10
Cr	205.56	Strong	Linear	None	0–10	0.999990	15
Cr	267.716	Strong	Linear	None	0–10	0.999992	10
Cr	276.653	Strong	Linear	None	0–10	0.999608	10
Cu	217.895	Intermediate	Quadratic	None	30–600	0.999631	10
Cu	222.778	Intermediate	Quadratic	None	30–600	0.999991	10
Cu	324.754	Strong	Quadratic	None	0–50	0.999999	10
Cu	327.395	Strong	Quadratic	None	0–30	0.999205	10
Fe	234.35	Intermediate	Quadratic	None	30–400	0.999995	10
Fe	238.204	Strong	Quadratic	None	0–10	0.999993	15
Fe	240.489	Strong	Quadratic	None	0–30	0.999433	10
Fe	259.837	Intermediate	Quadratic	None	10–600	0.999564	10
Mg	277.983	Intermediate	Quadratic	None	10–600	0.999774	15
Mg	278.297	Intermediate	Quadratic	None	30–600	0.999996	10
Mg	285.213	Strong	Quadratic	None	0–30	0.999811	10
Mg	383.829	Strong	Quadratic	None	0–200	0.999983	10
Mn	260.568	Strong	Quadratic	None	0–30	0.999702	10
Mn	261.815	Intermediate	Quadratic	None	30–600	0.999653	10
Mn	263.817	Intermediate	Quadratic	None	30–600	0.999825	15
Mn	293.931	Strong	Quadratic	None	0–50	0.999999	10
Mo	202.032	Strong	Quadratic	None	0–10	0.999998	15
Mo	204.598	Intermediate	Linear	None	0–10	0.999937	15
Ni	216.555	Strong	Quadratic	None	0–10	0.999978	15
Ni	222.486	Intermediate	Linear	None	0–10	0.999909	15
Ni	231.604	Strong	Quadratic	None	0–10	0.999998	15
Pb	220.353	Intermediate	Linear	Co	0–10	1.000000	15
Se	196.026	Weak	Linear	Fe	0–10	0.99909	15
Zn	206.2	Strong	Quadratic	None	0–50	0.999802	10
Zn	213.857	Strong	Quadratic	None	0–30	0.999996	15
Zn	334.502	Intermediate	Quadratic	None	30–600	0.999751	15
Zn	472.215	Intermediate	Quadratic	None	30–600	0.999575	10

[a] Error may exceed 10% for some of the lowest concentration standards.

Table 6. Comparison of results of certified and consensus values

	NIST SRM 695			Magruder 2009-06		
Element	Certified	Mean	Recovery, %	Consensus, ICP	Mean	Recovery, %
As, mg/kg	200 ± 5	199.9	100.0	330.58 ± 20.55	358.4	108.4
Cd, mg/kg	16.9 ± 0.2	17.1	101.2	343.55 ± 19.70	348.1	101.3
Co, mg/kg	65.3 ± 2.4	61.7	94.5	945.97 ± 53.68	959.3	101.4
Cr, mg/kg	244 ± 6	226.4	92.8	111.68 ± 11.16	127.9	114.6
Mo, mg/kg	20.0 ± 0.3	19.5	97.5	17.80 ± 2.70	18.1	101.7
Ni, mg/kg	135 ± 2	127.6	94.5	1135.8 ± 81.32	1117.3	98.4
Pb, mg/kg	276 ± 17	284.9	103.2	3688.5 ± 1852.4	4869.6	132.0
Se, mg/kg	2.1 ± 0.1[a]	1.6	74.6	116.46 ± 8.33	110.6	94.9
Ca, %	2.26 ± 0.04	2.28	102.5	1.78 ± 0.12	1.79	100.4
Cu, mg/kg	1225 ± 9	1214	99.1	334 ± 38	339.7	101.7
Fe, %	3.99 ± 0.08	3.98	99.7	2.03 ± 1.02	3.03	149.0
Mg, %	1.79 ± 0.05	1.76	98.2	0.18 ± .12	0.191	105.9
Mn, %	0.305 ± 0.005	0.311	101.9	0.153 ± 0.013	0.177	115.5
Zn, %	0.325 ± 0.005	0.317	97.7	0.165 ± 0.11	0.164	99.3

[a] Reference value.

standard solution into a 500 mL acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*10*) *100 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 10.0 mL of single element or combined 10 000 mg/L multielement stock standard solution into a 1000 mL acid-washed volumetric flask. Add 90 mL trace metal grade HNO_3 and 30 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*11*) *80 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 8.0 mL of single element or combined 10 000 mg/L multielement stock standard solution into a 500 mL acid-washed volumetric flask. Add 45 mL trace metal grade HNO_3 and 15 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*12*) *50 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 5.0 mL of single element or combined 10 000 mg/L multielement stock standard solution into a 1 L acid-washed volumetric flask. Add 90 mL trace metal grade HNO_3 and 30 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*13*) *30 mg/L Ca, Cu, Fe, Mg, Mn, and Zn.*—Pipet 3.0 mL of single element or combined 10 000 mg/L multielement stock standard solution into a 1 L acid-washed volumetric flask. Add 90 mL trace metal grade HNO_3 and 30 mL trace metal grade HCl, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(*14*) *0.0 mg/L all Ca, Cu, Fe, Mg, Mn, and Zn.*—Add 45 mL trace metal grade HNO_3 and 30 mL trace metal grade HCl into a 500 mL volumetric flask, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(p) *Sampler wash solution, 9% HNO_3:3% HCl.*—Dilute 90 mL trace metal grade HNO_3, G(b), and 30 mL trace metal grade HCl, G(c), to 1000 mL with H_2O.

H. Determination

Analyze test solutions using an ICP-OES instrument calibrated with standard solutions. Insert a 10 mg/kg working standard or other suitable quality control solution every 10 test portions to monitor for instrument drift. For quality control, *see* section J.

I. Calculations

$$Element, \frac{mg}{kg} = \left(instrument\ conc, \frac{mg}{L}\right) * \left(\frac{100\ mL}{sample\ wt., g}\right) * \left(\frac{1\ L}{1000\ mL}\right) * \left(\frac{1000\ g}{1\ kg}\right)$$

where 100 mL assumes the microwave digest is diluted to 100 mL.

Some of the Group A and B elements are routinely reported as percent concentrations. To convert a mg/kg result to percent, divide the mg/kg result by 10 000 and change the unit from mg/kg to percent.

J. Quality Control

Each run should contain adequate quality control to monitor the analytical system. The following are recommended to be included with each batch prepared for digestion.

(a) *Accuracy check.*—One or more digested reference materials of known concentration (e.g., NIST 695, Magruder Check Samples, AFPC Check Samples, etc.).

(b) *Precision check.*—One of the unknowns should be duplicated to ensure that the process can repeat a similar result.

(c) *Method blank.*—A digestion tube containing all reagents with no test portion that is processed identically to all others

Table 7. Method precision and comparability to 2006.03

Material	Proposed method (n = 3) Avg., mg/kg	RSD, %	Collaborative study 2006.03 results Avg., mg/kg	RSD, %	Recovery, %	HorRat (r)
			As			
A	41.49	1.86	22.15	42.93	187.32	0.29
B	478.89	1.39	263.2	47.96	181.95	0.31
C	6.89	3.05	4.87	63.12	141.42	0.36
D	5917.76	3.43	4945	6.09	119.67	1.12
E	2953.58	0.62	2432	10.56	121.45	0.18
F	10.36	2.10	9.75	41.73	106.26	0.27
G	22.32	0.35	22.43	9.36	99.49	0.05
H	2.92	3.32	2.36	17.60	123.63	0.35
I	11.95	1.77	13.04	13.27	91.63	0.23
J	189.46	2.23	185.45	4.27	102.16	0.43
K	168.49	2.23	175.28	7.84	96.13	0.43
L	BDL[a]	NA[b]	NA	NA	NA	NA
M	9.31	1.45	7.35	53.17	126.64	0.18
N	17.41	1.82	12.74	15.74	136.62	0.25
O	3.77	4.95	4.16	45.14	90.66	0.54
P	60.72	3.73	47.83	2.22	126.94	0.61
			Cd			
A	2.40	3.92	2.25	39.14	106.78	0.40
B	5.29	7.83	7.56	19.65	69.97	0.39
C	22.07	1.79	21.28	0.61	103.72	0.25
D	44.91	4.04	36.64	3.2	122.58	0.64
E	27.86	6.51	22.58	4.25	123.40	0.91
F	235.39	0.76	214.6	3.06	109.69	0.15
G	28.73	1.61	26.69	5.79	107.65	0.24
H	63.58	0.90	55.23	1.25	115.09	0.15
I	BDL	NA	NA	NA	NA	NA
J	16.65	0.44	15.52	2.77	107.26	0.06
K	66.05	2.23	64.04	2.95	103.14	0.38
L	BDL	NA	NA	NA	NA	NA
M	4.32	9.64	4.19	3.18	103.17	1.07
N	BDL	NA	NA	NA	NA	NA
O	BDL	NA	NA	NA	NA	NA
P	0.52	20.41	0.57	66.74	92.02	0.94
			Co			
A	119.71	2.69	97.75	4.12	122.47	0.49
B	212.27	0.89	185.6	9.34	108.52	0.18
C	BDL	NA	NA	NA	NA	NA
D	19.80	0.39	17.33	4	114.25	0.05
E	26.50	1.28	23.01	3.53	115.15	0.19
F	9.05	1.14	8.91	2.94	101.57	0.14
G	BDL	NA	NA	NA	NA	NA
H	BDL	NA	NA	NA	NA	NA
I	BDL	NA	NA	NA	NA	NA
J	59.57	0.39	45.2	8.52	131.79	0.08
K	545.71	3.30	532.78	2.45	102.43	0.64

Table 7. (*continued*)

Material	Proposed method (n = 3) Avg., mg/kg	RSD, %	Collaborative study 2006.03 results Avg., mg/kg	RSD, %	Recovery, %	HorRat (r)
L	BDL	NA	NA	NA	NA	NA
M	22.54	4.39	21.25	4.98	106.08	0.76
N	BDL	NA	NA	NA	NA	NA
O	BDL	NA	NA	NA	NA	NA
P	13.65	2.12	10.67	9.14	127.93	0.28
			Cr			
A	892.48	2.46	731.5	10.09	122.01	1.17
B	461.20	1.26	396.99	14.20	116.17	0.55
C	172.14	0.47	159.5	1.31	107.93	0.18
D	45.39	0.66	38.25	3.66	118.67	0.20
E	122.57	1.23	101.15	2.23	121.18	0.44
F	586.13	0.83	566.18	8.00	103.53	0.38
G	302.41	1.00	281.91	2.88	107.27	0.42
H	380.12	0.32	341.28	3.05	111.38	0.14
I	18.31	0.71	18.11	1.26	101.09	0.19
J	219.62	0.14	164.4	10.38	133.59	0.05
K	189.16	1.09	169.49	2.76	111.60	0.72
L	6.41	1.97	5.84	5.52	109.78	0.23
M	128.59	2.29	115.55	2.69	111.89	0.83
N	6305.07	2.55	5980.93	0.99	105.42	1.67
O	120.89	1.36	108.85	6.75	111.88	0.49
P	146.07	2.83	134.77	7.08	108.38	1.05
			Mo			
A	109.17	0.90	69.16	7.35	157.85	0.30
B	156.58	2.47	116.69	18.28	134.18	0.89
C	4.84	2.50	3.89	10.04	124.40	0.54
D	3.72	3.19	2.73	21.89	136.22	0.66
E	6.88	6.27	4.39	22.27	156.79	0.70
F	20.48	1.26	18.47	1.68	110.91	0.34
G	4.41	1.47	4.00	12.49	110.31	0.32
H	13.69	0.79	11.74	4.75	116.61	0.20
I	BDL	NA	NA	NA	NA	NA
J	19.39	0.36	13.21	7.94	146.76	0.09
K	44.68	2.10	42.88	5.23	104.19	0.65
L	BDL	NA	NA	NA	NA	NA
M	14.70	1.93	11.53	15.03	127.53	0.49
N	9.31	1.92	7.83	7.22	118.88	0.23
O	BDL	NA	NA	NA	NA	NA
P	15.53	0.76	12.44	14.46	124.83	0.20
			Ni			
A	384.29	0.57	331.92	3.94	115.78	0.36
B	330.30	3.66	295.83	18.02	111.65	0.55
C	30.10	3.63	26.60	3.55	113.14	0.37
D	BDL	NA	NA	NA	NA	NA
E	39.14	3.35	36.39	5.75	107.57	0.37
F	296.16	0.38	279.34	0.93	106.02	0.23

Table 7. (*continued*)

Material	Proposed method (n = 3) Avg., mg/kg	RSD, %	Collaborative study 2006.03 results Avg., mg/kg	RSD, %	Recovery, %	HorRat (r)
G	44.33	1.90	42.45	3.05	104.42	0.21
H	60.14	1.83	52.76	3.73	113.98	0.85
I	BDL	NA	NA	NA	NA	NA
J	122.10	0.67	101.89	7.03	119.84	0.35
K	1683.6	3.08	1683.27	4.63	100.02	0.59
L	BDL	NA	NA	NA	NA	NA
M	85.14	4.00	86.22	21.3	98.75	0.49
N	20.96	3.12	18.55	12.64	112.97	0.26
O	BDL	NA	NA	NA	NA	NA
P	36.90	3.89	33.39	13.42	116.56	0.42
			Pb			
A	136.03	8.63	119.6	64.13	113.74	1.60
B	3729	8.23	3070.11	30.44	121.47	0.60
C	1072	1.22	996.25	1.64	107.64	0.31
D	3790	0.47	3292.06	4.55	115.12	0.20
E	4121	4.04	4075.75	16.36	101.11	1.25
F	4.08	6.44	3.08	4.54	132.53	1.00
G	4.35	3.56	3.81	15.89	114.16	0.39
H	BDL	NA	NA	NA	NA	NA
I	BDL	NA	NA	NA	NA	NA
J	275.84	0.49	245.35	3.90	112.43	0.11
K	514.92	1.66	509.54	3.47	101.06	0.38
L	BDL	NA	NA	NA	NA	NA
M	70.73	0.24	66.29	17.21	106.69	0.04
N	62.25	7.02	58.53	6.55	106.36	1.16
O	3.25	13.07	3.34	6[illegible]	97.38	1.50
P	383.07	3.83	343.08	10.[illegible]3	111.66	0.83
			Se			
A	3.09	9.08	6.96	12.43	44.34	0.95
B	30.31	1.47	31.01	14.15	97.68	0.22
C	BDL	NA	NA	NA	NA	NA
D	34.70	4.01	28.4	1.71	122.17	0.61
E	26.73	1.37	25.9	11.05	103.20	0.20
F	7.10	6.93	1.[illegible]0	83.34	507.16	0.83
G	5.54	14.00	1.73	47.46	320.11	1.61
H	BDL	NA	NA	NA	NA	NA
I	BDL	NA	NA	NA	NA	NA
J	5.14	8.53	3.20	42.10	160.53	0.97
K	245.39	3.15	257.17	2.89	95.42	0.63
L	BDL	NA	NA	NA	NA	NA
M	9.16	10.35	9.47	8.57	96.70	1.28
N	BDL	NA	NA	NA	NA	NA
O	BDL	NA	NA	NA	NA	NA
P	BDL	NA	NA	NA	NA	NA

[a] BDL = Below detection limit.

[b] NA = Not applicable.

within the batch is recommended to ensure that no contamination of reagents, glassware, etc, has occurred.

(d) *Matrix spike recovery (optional).*—One of the unknowns or reference materials can be spiked with a known concentration of all elements to ensure that the matrix does not significantly reduce or enhance the recovery of the desired analyte.

(e) *Continuing calibration verification (CCV).*—A calibration standard run at periodic intervals (every 10th test solution) to verify the instrument is maintaining calibration.

(f) *Internal calibration verification (ICV).*—An undigested reference solution from a source different from the calibration standards is run after the calibration to check the accuracy of the calibration.

(g) For each element not reaching predetermined QC criteria, the instrument must be recalibrated and the impacted samples must be reanalyzed.

(h) Limits of quantitation and detection (LODs and LOQs) should be determined for each element by each laboratory using the method. The author's LODs and LOQs should be used only as a guide, but due to different instruments and configurations, these will vary from user to user.

(i) A typical analytical sequence is as follows: (*1*) Instrument calibration standards, (*2*) ICV, (*3*) a series of test solutions based on digestion batch size, including digested QC, spike blanks, and QC duplicates and spikes, (*4*) CCV, (*5*) another group of test solutions and periodic CCV until finished, (*6*) final CCV and QC. It is recommended that one or more QC samples be included after every 10 digests.

Experimental (Validation Experiments)

Calibration and Linearity

Calibrations for elements at wavelengths listed in Table 2 using the working standard concentrations in Table 3 were investigated. The most suitable type of calibration curve was determined (linear or quadratic) using the software provided by the instrument. Linearity was determined by comparing responses for the working standard solutions over the range of expected concentrations.

Trueness or Accuracy

Accuracy was determined by results of analysis for all elements of interest, Group A and Group B, in NIST SRM 695 Trace Elements in Multi-Nutrient Fertilizer. Additionally, the results by the proposed method were compared to consensus values for Magruder 2009-06, and expressed as percentage recovery of the certified or consensus value.

Precision

Method precision was determined by independent analysis of the validation materials in triplicate, and variation was expressed as RSD.

Comparability

Comparability to AOAC **2006.03** for Group A elements was established by testing 15 of the original collaborative study

Table 8. Method precision for Ca, Cu, Fe, Mg, Mn, and Zn

	Ca			Cu			Fe			Mg			Mn			Zn		
ID	Mean, % (n = 3)	RSD, %	HorRat (r)	Mean, mg/kg (n = 3)	RSD, %	HorRat (r)	Mean, % (n = 3)	RSD, %	HorRat (r)	Mean, % (n = 3)	RSD, %	HorRat (r)	Mean, mg/kg (n = 3)	RSD, %	HorRat (r)	Mean, % (n = 3)	RSD, %	HorRat (r)
A	0.869	1.07	0.26	6636.7	0.58	0.38	53.76	4.49	0.24	0.240	0.81	0.16	15065	1.22	1.30	1.506	2.51	1.33
B	2.497	0.93	0.27	9123.1	1.33	0.99	24.95	2.58	1.05	1.102	0.74	0.19	63109	1.07	1.41	5.371	0.79	0.51
C	0.510	1.94	0.44	1547	1.75	0.62	4.458	0.28	0.23	0.343	0.92	0.20	6251.7	0.58	0.54	36.57	0.91	0.78
D	3.411	1.42	0.43	14264	1.88	0.80	13.96	3.67	1.37	1.964	1.23	0.34	780.77	1.36	0.93	1.265	0.96	0.50
E	2.797	1.57	0.46	9767.8	0.65	0.29	10.59	2.86	1.10	1.728	0.84	0.23	10646	1.17	1.16	4.115	1.00	0.62
F	0.913	0.96	0.24	5421.2	1.03	0.12	1.074	0.74	0.19	0.652	0.90	0.21	227.94	0.81	0.46	0.287	1.18	0.49
G	1.418	0.57	0.15	684.31	0.52	0.08	0.241	0.45	0.18	0.377	0.51	0.11	25.76	1.14	0.46	0.0424	0.74	0.23
H	0.378	0.42	0.09	589.86	8.32	0.75	1.417	0.30	0.15	1.379	0.58	0.15	146.61	0.35	0.19	0.098	0.78	0.28
I	0.149	2.81	0.53	498.88	1.98	0.19	0.62	0.33	0.16	1.044	0.65	0.16	217.12	0.28	0.16	0.0176	1.01	0.28
J	2.169	1.36	0.38	368.99	1.85	0.94	3.805	1.52	0.47	1.657	1.34	0.36	2835.0	2.09	1.73	0.302	1.36	0.57
K	4.580	2.35	0.74	370.35	0.70	0.33	2.268	1.13	0.42	1.350	1.91	0.50	2084.3	2.36	1.86	0.356	4.16	1.79
L	0.109	4.13	0.74	1800.0	1.86	0.20	2.103	1.33	0.18	0.168	0.65	0.12	167.15	1.26	0.68	0.0046	2.87	0.64
M	1.724	0.52	0.14	3192.9	1.04	0.42	4.045	0.89	0.23	0.568	1.11	0.26	269.41	2.61	1.52	0.0568	1.08	0.35
N	3.976	1.29	0.40	4651.4	2.39	0.93	8.927	1.15	0.40	0.387	0.55	0.12	1618.5	0.11	0.08	0.0353	1.95	0.59
O	2.493	1.88	0.54	3214.1	3.12	0.44	0.23	2.00	0.80	0.208	0.79	0.16	114.46	3.49	1.78	0.0085	1.70	0.42
P	6.511	0.39	0.13	1757.3	1.80	0.30	23.82	3.95	1.59	0.412	1.10	0.24	9625.0	1.00	0.99	0.0065	2.22	0.52

Table 9. Group A instrument and method LOD and LOQ

	mg/L							
	As	Cd	Cr	Co	Mo	Ni	Pb	Se
Instrument LOD	0.10	0.048	0.038	0.036	0.10	0.0006	0.0007	0.0003
Instrument LOQ	0.36	0.17	0.13	0.13	0.36	0.0023	0.0023	0.0011
Method LOD	0.57	0.11	2.13	0.97	0.15	0.49	0.59	0.45
Method LOQ	2.03	0.39	7.55	3.43	0.53	1.74	2.09	1.60

materials with both digestion methods. Data are expressed as a percentage recovery of the original grand average result.

Range

The working range of the method is determined by the working calibration standards listed in Table 3. Each laboratory should determine the range best suited to their instrument's capability. In general, 10% extrapolation from the highest calibration standard shown in Table 3 often produces acceptable results for Group A and B elements. Preferably, any analyte that exceeds the instrument response should be diluted so that the instrument response falls between the upper and lower calibration standards and so that the matrix of 9% HNO_3 and 3% HCl is maintained.

LOD

The LOD was determined two ways. The instrument LOD was determined by running 10 reagent blanks. The method LOD was determined by analyzing an N-P-K blend known to contain the Group A elements below the suspected level of detection 10 separate times. The SD of the results was multiplied by 2.821 to determine the LOD, and the LOQ was determined by multiplying the LOD times 3 (8).

Method Ruggedness Tests

Optimization testing was conducted using the Youden ruggedness trial (6) to determine the effects of seven variables: (*1*) acid ratio, (*2*) test portion, (*3*) digestion time, (*4*) digestion temperature, (*5*) volume of dilution, (*6*) filtration, and (*7*) dilution method. The major and minor criteria are listed in Table 4.

Results

Calibration and Linearity

Calibrations were evaluated and results are provided in Table 5. All results reported have undergone corrective calculations, in the instrument manufacturer's software Section I, to include the test portion weight and total volume to yield the corrected values.

Trueness or Accuracy

Results obtained for NIST SRM 695 were compared with the certified or reference values, and results obtained for Magruder 2009-06 were compared to consensus values. Percent recovery for As, Cd, Co, Cr, Mo, Ni, Pb, Se, Ca, Cu, Fe, Mg, Mn, and Zn for NIST SRM 695 were 100, 101, 94.5, 92.8, 97.5, 94.5, 103.2, 74.6, 102.5, 102, 99.1, 99.7, 102, 97.7, respectively, and for Magruder 2009-06 were 108, 101, 101, 115, 102, 98.4, 132, 94.9, 100, 102, 149, 106, 115, and 99.3, respectively. Results are found in Table 6.

Precision

Results of precision experiments are found in Tables 7 and 8.

Comparability

Results of comparability experiments are found in Table 7.

LOQ and LOD

The LOQ and LOD for Group A elements and the practical LOQ (LOQ) for Group B were found to be as provided in Tables 9 and 10. Table 9 provides the instrument LOD (estimated with standard solutions) and the method LOQ and LOD (estimated with validation materials) for the Group A metals. The LOQ for Group B elements are listed in Table 10 are completely dependent upon the calibration range since the method is not working close to the instrument limits. Lower limits for Group B elements can be achieved by altering the test portion size or choosing more sensitive wavelengths. Table 10 presents LOQ's for Group B metals based on the calibrations as suggested in the proposed method.

Method Ruggedness Tests

Optimization testing was conducted using the Youden ruggedness trial (6) to determine the effects of seven variables: (*1*) acid ratio, (*2*) test portion, (*3*) digestion time, (*4*) digestion temperature, (*5*) volume of dilution, (*6*) filtration, and (*7*) dilution method. The major and minor criteria are listed in Table 4. Results are provided in Table 11. The differences in Table 11 are all within normal variation and indicate that the method is sufficiently rugged with respect to the conditions studied.

The Se values in the sample are at or below the method LOQ, so the values obtained are not accurate. Also, as noted in the interlaboratory study (*see* Appendix) Fe is the most variable element. So, the RSDs obtained for Fe in the ruggedness study are a little higher than the other elements as is expected. This represents an advanced ICP-OES method, so familiarization, practice, and demonstration of proficiency before use is strongly recommended. Also, since ICP-OES instruments differ in design,

Table 10. Practical LOQ for group B metals

	Ca, %	Cu, mg/L	Fe, %	Mg, %	Mn, %	Zn, %
Practical LOQ	0.0045	0.49	0.0018	0.0015	0.00003	0.0009

Table 11. Ruggedness tests results

		Average of major values—average of minor values						
Metal	Result	Acid ratio[a]	Test portion[a]	Digestion time[a]	Digestion temp.[a]	Dilution vol.[a]	Filtering	Dilution technique
As, mg/kg	199.9	–0.64	7.80	1.46	–2.29	1.43	1.96	–2.05
Cd, mg/kg	17.1	–0.15	0.50	0.048	–0.45	–0.36	0.44	0.031
Co, mg/kg	61.7	–4.17	0.35	–4.18	1.39	1.9	0.70	–4.97
Cr, mg/kg	226.4	–6.32	7.42	1.91	0.76	3.35	9.10	–7.09
Mo, mg/kg	19.5	–0.94	1.98	1.67	–1.58	–0.85	0.076	0.001
Ni, mg/kg	127.6	6.29	–0.033	–0.22	1.43	0.74	–2.41	0.85
Pb, mg/kg	284.9	4.81	–0.59	0.50	2.52	1.38	0.38	–0.76
Se, mg/kg	1.6	0.56	–0.26	–0.20	0.26	0.24	–0.22	0.22
Ca, %	2.28	–0.022	0.093	0.013	–0.0080	–0.010	0.040	–0.013
Cu, ppm	1214	–9.67	30.60	16.46	–26.15	0.83	11.0	–13.39
Fe, %	3.98	0.12	0.46	0.39	–0.10	0.15	0.41	0.072
Mg, %	1.76	0.0058	0.010	0.014	–0.020	0.013	–0.0018	–0.0014
Mn, %	0.311	0.0070	0.0033	0.0013	0.0021	–0.0018	–0.00043	–0.0032
Zn, %	0.317	0.011	0.0057	–0.0082	–0.0030	0.0015	–0.0070	–0.0029
		Average of major values—average of minor values expressed as RPD[b]						
As, mg/kg	199.9	0.32	3.90	0.73	1.14	0.72	0.98	1.01
Cd, mg/kg	17.1	0.88	2.92	0.28	2.63	2.11	2.57	0.18
Co, mg/kg	61.7	6.76	0.57	6.77	2.25	3.08	1.13	8.06
Cr, mg/kg	226.4	2.79	3.28	0.84	0.34	1.48	4.02	3.13
Mo, mg/kg	19.5	4.82	10.2	8.56	8.10	4.36	0.39	0.005
Ni, mg/kg	127.6	4.93	0.026	0.17	1.12	0.58	1.89	0.67
Pb, mg/kg	284.9	1.69	0.21	0.18	0.88	0.48	0.13	0.27
Se, mg/kg	1.6[c]	35.0	16.3	12.5	16.25	15.0	13.75	13.7
Ca, %	2.28	0.96	4.08	0.57	0.35	0.44	1.75	0.57
Cu, ppm	1214	0.80	2.52	1.36	2.15	0.068	0.90	1.10
Fe, %	3.98	3.02	11.56	9.80	2.51	4.02	10.30	1.81
Mg, %	1.76	0.33	0.57	0.080	1.14	0.74	0.10	0.080
Mn, %	0.311	2.25	1.06	0.42	0.68	0.58	0.14	1.03
Zn, %	0.317	3.47	1.80	2.59	0.95	0.47	2.21	0.91

[a] Values in table are the difference between the major and minor variable, or RPD; units for the variables are listed in Table 4.

[b] RPD = Relative percent difference.

[c] <LOQ.

introduction system, plasma efficiency, viewing orientation, RF generation, etc., some minor adjustments to the instrument conditions may be necessary. Any adjustments must be performance based, using a variety of known reference materials over a wide range of analyte concentrations.

Conclusions

The proposed method is suitable for the simultaneous determination of Group A (As, Cd, Co, Cr, Pb, Mo, Ni, and Se) and Group B (Ca, Cu, Fe, Mg, Mn, and Zn) metals in fertilizers with digestion by microwave mixed acid digestion and detection by ICP-OES. It meets the criteria recommended by the AAPFCO Laboratory Services Committee. This method is proposed for adoption as a regulatory method and for further collaborative study.

References

(1) Kane, P., & Hall, W. (2006) *J. AOAC Int.* **89**, 1447–1466
(2) *Official Methods of Analysis*, 18th Ed. (2005) AOAC INTERNATIONAL, Gaithersburg, MD, Method **2006.03**
(3) Association of American Plant Food Control Officials (AAPFCO) Official Publication, No. 66 (2013) p. 66
(4) Webb, S., Bartos, J., Boles, R., Hasty, E., Thuotte, E., & Falls, H. (2009) *Simultaneous Determination of Nutritive and Nonnutritive Metals in Fertilizers by ICP-OES*, September 2009, Proceedings, Abstract 1804, 123rd AOAC INTERNATIONAL Annual Meeting and Exposition, Philadelphia, PA
(5) EPA Method 3051A, *Microwave Assisted Microwave Digestion of Sediments, Sludges, Soils and Oils* (2007) U.S. Environmental Protection Agency, Cincinnati, OH. www.epa.gov/region9/qa/pdfs/40cfr13603.pdf, p. 31 (accessed May 23, 2013)
(6) Youden, W.J., & Steiner, E.H. (1975) *Statistical Manual of the AOAC*, AOAC INTERNATIONAL, Gaithersburg, MD, pp 33–36

II. SINGLE LABORATORY VALIDATION:

1. Method Protocol in AOAC format.

The proposed method in AOAC format is found in the attached manuscript, Appendix 2 ("Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry Detection: Single-Laboratory Validation of a Modification and Extension of AOAC 2006.03), beginning in the second column on page 2 and ending in the second column on page 9.

2. Method Validation / Method Performance:

The attached manuscript, Appendix 2, has been peer reviewed and published in JAOAC. It describes the single laboratory validation in detail.

II. RECOMMENDATION:

Study Director(s): Sharon Webb

Proposed ERP Members:

Patty Lucas, Harold Falls, Dennis Sebastian, Victoria Siegel, Lawrence Novotny, and Frank Sikora.

IV. STUDY DESIGN PROPOSAL

1. Scope/applicability:

This method is applicable to the determination of arsenic (As), cadmium (Cd), calcium (Ca), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe),lead (Pb), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn) in all classes of fertilizers. This is an extension and modification to AOAC Method 2006.03 by extending the analytes to include micronutrients (Ca, Cu, Fe, Mg, Mn, and Zn) and adding hydrochloric acid to the digestion to increase recoveries of several metals. For ease of presentation and discussion of results, the metals are broken down into two groups. "Group A metals" are all the metals referred to in AOAC 2006.03 (As, Cd, Cr, Co, Pb, Mo, Ni, and Se). The proposed elements to be added to the scope will be referred to as "Group B metals" (Ca, Cu, Mg, Mn, and Zn).

2. Materials/matrices:

The validation materials consist of test materials from the previous collaborative study that adequately represent all classes of fertilizer ingredients and fertilizer blends in a wide range of concentrations of all analytes. There are 5 fertilizer classes represented: micronutrient mixes, concentrated phosphate products, N-P-K blends, organic materials, and phosphate ores. Magruder 2009-06 and NIST SRM 695 will be included as test materials to be used to assess the recovery (i.e. accuracy/bias) and reproducibility of the proposed method. Each analyte is represented in high, medium, and low concentration as seen in Tables 1C and 2C.

Table 1C: Concentration Ranges of Group A Metals

Metal	High Concentration	Medium Concentration	Low Concentration
As,(ppm)	5900	190	2.03
Cd,(ppm)	235	66	0.39

Co,(ppm)	546	26	0.97
Cr,(ppm)	6300	220	2.13
Mo,(ppm)	157	45	0.53
Ni,(ppm)	1684	296	1.74
Pb,(ppm)	4121	136	2.09
Se,(ppm)	245	30	1.60

Table 2C: Concentration Ranges of Group B Metals

Metal	High Concentration	Medium Concentration	Low Concentration
Ca, (%)	6	0.9	0.0045
Cu,(ppm)	14000	1.33	0.49
Fe, (%)	54	0.241	0.0018
Mg, (%)	2	0.4	0.0015
Mn, (%)	15000	147	0.00003
Zn, (%)	36	0.3	0.0009

3. Test sample preparation and handling:

The solid test materials were prepared using AOAC method **929.02**. The unground sample was ground using a Model MC 200 Electric Miracle Seed & Coffee Mill. Once ground, the material was passed through a 40 mesh sieve, and any remaining +40 mesh sieve was reground until it passed through the 40 mesh sieve. The entire ground sample was then rolled 125 times. Then a spatula was used to "cut" the sample to break up any lumps of material tending to clump. This procedure was performed 4 times until the sample had been rolled 100 times. The material was then placed in the middle of the rolling cloth, and individual ground sample portions were extracted from random locations in the pile using a small weighing spatula. This is the same procedure used to prepare proficiency samples for the Magruder and Rock Check Programs.

Approximately 5 g sub-samples will be placed in 20 mL glass vials for distribution to participants. The sixteen samples used in the single laboratory validation will be distributed in blind duplicates. Therefore a total of 18 samples, including NIST SRM 695, Magruder 2009-06, and a method blank sample (Boric Acid) which has been specially formulated to contain a high concentration of Group A metals.

The study samples are given a unique identification and then put into a spreadsheet to randomize the distribution of the blind duplicates. So to minimize confusion for the collaborators, the individual samples will only contain sequential number identifiers from the order contained in the randomized key.

Table 3: Study samples for extension and modification of 2006.03

ID	ID from 2006.03 Collaborative Study	Description
A	4321/2025	Metal Fe oxysulfate
B	4031/5938	Magruder 2002-09B (micronutrient mix)
C	5488/5890	Zinc oxysulfate

D	2818/7669	Granulated mine waste
E	1615/2056	Metal oxysulfate
F	3313/6267	Western MAP
G	7999/3375	DAP from North African rock
H	6501/4812	NC MAP
I	7738/7418	China DAP
J	3917/8165	Magruder 2003-11 (14-14-14 blend)
K	4459/8931	Magruder 2004-07 (10-10-10)
L	8873/9469	N-P-K lawn product blend
M	9886/9774	Organic biosolid
N	4626/8088	Organic mixed fertilizer + biosolid
O	6411/3401	Composted manure
P	3716/4606	Fe humate
Q	NA	NIST SRM 695
R	NA	Magruder 2009-06 (12-12-12)

The samples have demonstrated homogeneity from their use in the 2006.03 collaborative study. The NIST SRM 695 and the Magruder 2009-06 samples will not be tested for homogeneity either, as their homogeneity has been demonstrated as well.

Fertilizer samples have a known stability well beyond the test window assigned to this study.

Test materials will be sealed and carefully packaged into shipping containers, and the test materials, cover letter, instructions, a SOP, and data recording sheets will be shipped via carriers to the contact person at the address provided by the collaborating laboratory.

4. Quality Assurance

Practice samples will be provided to collaborators as part of the pre-trial method familiarization process. These samples are identified in Table 4 and will include certified reference/accuracy materials, and representation of fertilizer types and matrices with low, medium, and high concentrations of the metals in the study.

Table 4: Proposed materials to be used in the familiarization study

	Source	As, ppm	Cd, ppm	Co, ppm	Cr, ppm	Mo, ppm	Ni, ppm	Pb, ppm	Se, ppm
12-12-12	M 2009-06	333.85	345.16	958.74	108.8	17.65	1153.3	3815.4	116.01
Micros	M 2007-03	33.8	4.67	41.33	63.06	575.68	5394.6	49.72	16.15
0-46-0	AFPC08-07B	29	100	2	364	5	38	5	5
Cu-Zn Mix	M 2008-10	4.71	1.86	50.9	214.94	28.38	149.45	101.79	6.7

46-0-22	**M 2008-01**	2.87	0.44	3.83	117.8	12.08	11.15	11.12	0
0-0-0 MEM	**M 2006-04**	184.88	12.86	83.59	158.38	20.78	77.12	681.24	36.46
5488/5890	**2006.03**	4.87	21.28	4.79	159.5	3.89	26.6	996	4.62
4321/2025	**2006.03**	22.15	2.25	97.8	731.4	69.16	331.9	119.6	6.69
Boric Acid (method blank)	**NIST**	0	0	0	0	0	0	0	0
NIST 695	**NIST**	200.3	16.92	65.3	244	15	134.9	273	2.12

Table 5. Group A Instrument and Method Detection and Quantitation Limits.

	As, mg/L	Cd, mg/L	Cr, mg/L	Co, mg/L	Mo, mg/L	Ni, mg/L	Pb, mg/L	Se, mg/L
Instrument LOD	0.10	0.048	0.038	0.036	0.10	0.0006	0.0007	0.0003
Instrument LOQ	0.36	0.17	0.13	0.13	0.36	0.0023	0.0023	0.0011
Method LOD	0.57	0.11	2.13	0.97	0.15	0.49	0.59	0.45
Method LOQ	2.03	0.39	7.55	3.43	0.53	1.74	2.09	1.60

Table 6. Group B Practical Quantitation Limits.

	Ca, %	Cu, mg/L	Fe, %	Mg, %	Mn, %	Zn, %
Practical LOQ	0.0045	0.49	0.0018	0.0015	0.00003	0.0009

Based upon the result of the practice samples,, the limit of detection from the method blank will be recalculated and assessed. The above tables (Tables 5 and 6) are the results based on the single laboratory validation.

The results from the practice samples will be evaluated by three criteria to determine the performance of the collaborating laboratory. First, the results obtained by the collaborating laboratory will be compared to the values from the certified reference and check sample materials to verify they are within or very near the prescribed variability for all analytes of interest. Second, the results will be assessed to verify that they fall within two standard deviations from all collaborating laboratories. Third, the recoveries will be calculated and checked that they fall within the recommended recovery limits as seen in Table 7. Laboratories that exceed in more than one of these criteria will be notified and if they still wish to participate, the Study Director will work closely with the lab to attempt to identify and resolve the source of variability.

The results from the analysis of the practice samples will be used to determine if the laboratory can meet the suitability requirements and proceed to the final study stage of the process.

5. Proposed approach to data analysis.

After any Cochran or Grubbs outliers are identified and removed, the percent recoveries from the certified reference and check sample materials will be calculated. The recoveries will be compared with those recommended for SLV studies, which are listed in Table 7 below.

Table 7. Recommended recovery limits for single lab validation:	
Concentration	**Recovery (%)**
100%	98 – 101
10%	95 – 102
1%	92 – 105
0.1%	90 – 108
0.01%	85 - 110
10 µg/g (ppm)	80 - 115
1 µg/g (ppm)	75 - 120

Calculations for repeatability (s_r) and reproducibility (s_R), as defined by the AOAC guidelines found in the *Official Methods of Analysis, 18th Edition (2005), Appendix D: "Guidelines for Collaborative Study Procedures to Validate Characteristics of a Method of Analysis."* HORRAT values will be calculated, with recommended HORRAT(r) repeatability values between 0.3 and 1.3 and HORRAT(R) reproducibility values between 0.5 and 2.0 for overall method assessment.

6. Other participating organizations.

The collaborative study has been planned and organized by Dr. Sharon F. Webb from the University of Kentucky, Division of Regulatory Services. Sample preparation assistance was received from Bill Hall with Mosaic. Technical support was provided by Nancy Thiex with Thiex Laboratory Solution, and financial support was provided by The Fertilizer Institute (TFI) and several fertilizer manufacturers.

Current organizations and laboratories that have expressed an interest in participating include: the North Carolina Department of Agriculture (State Regulatory), Oregon Department of Agriculture (State Regulatory), Milwaukee Metro Sewage Dist. Milorganite (Industry Laboratory), CEM Corporation (Instrument Vendor), Mosaic (Industry Laboratory), Florida Department of Agriculture and Consumer Services (State Regulatory), Agilent (Instrument Vendor), the Canadian Food Inspection Agency (International Regulatory), Thornton Labs (Private Laboratory), Office of the Indiana State Chemist (State Regulatory), Fritz Industries (Industry Laboratory), Georgia Department of Agriculture (State Regulatory), the University of Missouri (State Regulatory), EDW C. Levy Company (Industry Laboratory), California Department of Food and Agriculture (State Regulatory), Ohio Department of Agriculture (State Regulatory), Spectro Analytical Instruments Inc. (Industry Vendor), Washington Department of Agriculture (State Regulatory), Office of the Texas State Chemist (State Regulatory), Montana Department of Agriculture (State Regulatory), New York Department of Agriculture (State Regulatory),

Simplot (Industry Laboratory), PCS Phosphate Inc. (Industry Laboratory), and Nachurs Alpine Solutions (Industry Laboratory).

7. Estimated number of collaborators.

As outlined above to date 23 laboratories have expressed an interest in participating in this collaborative study. The current composition of the collaborators is as follows: 12 state regulatory laboratories, one international regulatory laboratory, 7 fertilizer industry laboratories, two ICP-OES instrument manufacturers, and one private laboratory. If necessary, some laboratories have multiple ICP-OES instruments and experienced chemists, so the study could be performed twice independently by the same laboratory.

The primary selection criteria for participation in the collaborative study include the following: an ICP-OES instrument, an experienced operator familiar with configuring methods, chemists familiar with testing fertilizer or other agricultural products and laboratories that can document the quality of their data through their participation in check sample programs, proficiency programs or other laboratory certifications.

8. Communication to collaborators.

The following cover letter will be distributed to colleagues and contacts who have expressed an interest in participating in this study:

Division of Regulatory Services
University of Kentucky
103 Regulatory Services Bldg.
Lexington, KY 40546-0275
USA

November 1, 2014

Dear Colleague,

I have been appointed the Study Director of an AOAC collaborative study of a method for the determination of several nutrient and nonnutrient metals in fertilizers using a mixed-acid microwave digestion and analysis by ICP-OES. I am writing to formally invite your laboratory to participate in this collaborative study, as we have previously communicated. At that time you expressed an interest in participating.

The single laboratory validation method has been published in the Journal of the AOAC International Volume 97, No. 3, 2014. The collaborative study is planned for 2014 and consists of 2 phases. The first phase will involve the analysis of a set of pre-study practice samples using the method. These samples will be used to verify shipping arrangements, to allow laboratories to familiarize themselves with the method and to work out any technical issues with the method, which may arise during the familiarization timeframe. The results from the pre-study practice samples should be submitted to the study director for evaluation. If necessary, technical recommendations

will be made to the individual laboratories to further refine their analysis to ensure validity of the final study results. The second phase of the study will involve the analysis of 36 study samples. This second phase will finalize the performance parameters of the nutrient and nonnutrient metals in fertilizers by microwave digestion and analysis via ICP-OES method. If the results meet the requirements of AOAC, the method will be published in the Journal of AOAC International and the Official Method of Analysis compendium.

Accompanying the study samples are several relevant documents. First is this cover letter and instructions. Second is the method SOP. Please note that it is critical to the success of the study that you follow the method exactly as written, without any modifications. If you are unable to attain the recommended chemicals, standards, supplies or instrument settings listed, please inform the Study Director prior to commencement of analyses. Also include this information in the comments section provided when reporting your results. Third is a *Data Record Sheet* on which you should record the weight of each sample and your final results. If you are missing any of these documents, or have any questions, please contact the Study Director.

Stability testing has indicated that the samples will be stable for the duration of the study period when stored at room temperature (~25 °C/75 °F). To address any settling and to ensure sample homogeneity, it is recommended that the sample vials be rolled on a bench top and that the liquid samples be shaken prior to taking a subsample portion.

The fertilizer samples are composed primarily of salts, so a lab coat, safety glasses and acid resistant gloves are recommended for protection. As outlined in the SOP the method requires the use of concentrated acids which should only be used in the fume hood. Allow the samples after digestion to cool for at least 30 minutes prior to uncapping and transferring the digestate to the volumetric flask and bringing to volume. Be sure to follow all safety recommendations provided in the MSDS and your laboratory chemical hygiene plan. Solutions generated from this method are considered hazardous waste due to the high acid content and should be labeled as such and disposed in the manner as proscribed by your chemical hygiene plan.

Some hard copies of the data are required for archival purposes. Record your sample weights and your final results for the practice samples (PS) and study samples (SS) on the "Data Record Sheet" (see Attached). On the Data Record Sheets, you will find a comments section where you can record any information or exceptions that will be evaluated by the Study Director. While an electronic or hard copy of the data does not need to be reported to the Study Director, it is recommended that a hard copy of the data be archived for seven years. If this is impractical, you may print a copy of your ICP-OES sample results, which can be archived by the Study Director.

Please report your practice study results by January 15, 2015 and your study sample results by December 31, 2015 to Sharon Webb.

Your contribution to this collaborative study is highly valued and appreciated. Should you have any questions or concerns, please contact the Study Director.

Respectfully,

Sharon F. Webb, Ph.D.
Study Director
University of Kentucky
Division of Regulatory Services
Email: sharon.webb@uky.edu
Phone: 859-218-2451

SIGNATURES AND CONTACT INFORMATION FOR STAKEHOLDERS

See Attached

Appendix

Background – Work leading to Single Laboratory Validation

In 2008, a small inter-laboratory study involving four laboratories was undertaken by a group of regulatory and industry chemists, led by the Office of Indiana State Chemist (1). The goals of the study were to determine the viability of a single universal method for the digestion and analysis of fourteen metals in fertilizers, and to evaluate the possible enhancement of recoveries by using a dual acid digestion system versus a single acid. One laboratory, the Missouri Experiment Station, performed AOAC Method 965.09 (2) to yield results for the Group B metals in fertilizer. The Office of Indiana State Chemist performed AOAC Method 2006.03 (3) to yield results for the Group A metals in fertilizer. The Florida Department of Agriculture and Consumer Services performed a hot block digestion of the test portions using nitric and hydrochloric acids by EPA 3050B (4) digestion. The CEM Corporation provided digestates via microwave digestion using the proposed dual acid method. All microwave and hot block digests were analyzed at the Office of Indiana State Chemist using matrix matched calibration standards and ICP-OES. This study compared AOAC 965.09 results, for the Group B metals and AOAC 2006.03 (5) results for the Group A metals against results from hot block and microwave mixed acid digestions and ICP-OES detection. AOAC 965.09 utilizes hot plate HCl digestion with atomic absorption detection. AOAC 2006.03 utilizes HNO_3 microwave digestion followed by ICP-OES detection. EPA 3050B utilizes a HNO_3, H_2O_2 and HCl hot block digestion followed by ICP-OES detection. The proposed modification and extension of AOAC 2006.03 utilizes HNO_3 + HCl digestion with ICP-OES detection. Nine study materials from the Magruder Check Sample Program (5) and five commercial fertilizer materials were used as validation materials to cover a dynamic range of metal concentrations and reagent grade potassium chloride was included as a method blank. Values for the validation materials used for the digestion and method comparison part of this study are listed in Tables A1 and A2.

Preliminary Studies on Group B Metals:

The results for the Group B metals using the proposed nitric and hydrochloric acid microwave digestion and ICP-OES detection were compared to the published Magruder Check Sample "Method Group Grand Average" consensus results. For the Group B metals 78% of 108 data points were within 1 standard deviation and 91% were within 2 standard deviations. For the results that exceeded two standard deviations of the "Method Group Grand Average," all were higher concentrations than the published values suggesting enhanced recoveries with the two acid mixture. Examination of the Group A metals results demonstrated that 63% of the 137 data points were within 1 standard deviation and 90% were within two standard deviations. Again, for the results that exceeded two standard deviations of the Magruder "Method Group Grand Average" all were higher than the published values suggesting enhanced recoveries with the two acid mixture. All data showed a reasonably normal distribution, with a slightly higher percent outside the two standard deviation window. The results showed that simultaneous digestion and detection of both groups of metals in fertilizers is viable and indeed enhanced recoveries for Group A and Group B metals in some fertilizer compounds is possible when compared to the single acid digestions. (3)

Results obtained from AOAC 965.09 were used as the basis of comparison for statistical analysis of the Group B elements. Results obtained from AOAC 2006.03 were used as the basis of comparison for statistical analysis of the Group A elements. The statistical analysis for the Group B analytes when comparing the results from AOAC 965.09, AOAC 2006.03, hot block mixed acid (HB-Mix), and microwave mixed acid digestions (MW-Mix), showed no significant difference in results for Ca, Cu, Mn and Zn ($P = 0.05$), suggesting any of these methods will produce comparable results.

In the case of Mg, the statistical results indicated no significant difference between AOAC 965.09 and 2006.03 results; however, when comparing the results from AOAC 965.09 with the mixed acid microwave and the mixed acid hot block methods, the results from the mixed acids were significantly higher at a P level of 0.05, but not highly significant at a P level of 0.01.

To determine if this difference could be attributed to the dual acid system, results from the single acid 2006.03 method were compared with results from the proposed dual acid microwave digest. At a P level of 0.05, the difference in Mg results was significant, but not highly significant (P = 0.01). The affect of the hydrochloric acid can be seen by an enhanced recovery of Mg. This is consistent with the application note in EPA Method 3051A (6) which states that Mg is one of the elements that typically requires the addition of hydrochloric acid to achieve adequate recovery.

Statistical analysis of the Fe data revealed that between the three alternative methods being considered for use, the microwave mixed acid system had the best agreement with AOAC 965.09. The method that produced Fe results that were most different from AOAC 965.09 was 2006.03. This can be attributed to the absence of hydrochloric acid in the 2006.03 method, which can cause a low Fe recovery. As is the case with Mg, Fe is another element which EPA Method 3051A states that hydrochloric acid is recommended for better recovery. Overall, there was good statistical agreement between the proposed mixed acid microwave digestion method and the official 965.09 method.

The enhanced recoveries of Fe using both nitric and hydrochloric acid during digestion are demonstrated in Figures A1, A2 and A3. In Figure A1, the difference in Fe results for the methods employed in this inter-laboratory study, for fertilizers containing less than 2%, is shown. For materials containing less than 0.5% Fe, there was relatively good agreement among methods. Once the amount of iron exceeded about 1.0%, the agreement across all methods declined. Sample 12 contained considerable Fe derived from an organic material, so it is not a valid matrix for the AOAC 965.09 part C method. Nitric acid would be required to adequately recover any Fe linked with an organic source. Figure A2 shows the recovery of iron for materials containing mid-level concentrations ranging from 5 to 10% Fe. Overall, there is good agreement among the methods except for 2006.03, which is the only method that does not contain any hydrochloric acid. This suggests this method would not fully recover Fe. Figure A3 shows results for materials containing high-level Fe concentrations in the range of 10 to 50% Fe, and demonstrates the most variability in recovery. The method that yielded the lowest Fe recovery was 2006.03. There is also

low recovery from the hot block mixed acid digestion across all high-level Fe materials. Only the official method 965.09 demonstrated good recovery for the 50% Fe containing material (Figure A3). Based upon the results presented in Figure A3, some modifications to the proposed mixed acid microwave method, such as a lower test portion weight, are needed to be explored to ensure sufficient recovery of Fe in materials that contain over 20% Fe.

Preliminary Studies on Group A Metals:

Statistical analysis of the Group A metals results was performed. AOAC method 2006.03 served as the basis for comparison against results obtained with both the proposed microwave mixed acid digestion (MW-Mix) and the hot block mixed acid digestion (HB-Mix) methods.

Comparison of Microwave Mixed-Acid and AOAC 2006.03 Data:

For As, Cd, Pb, Ni, and Se, at the P = 0.05 level, there was no difference between the values obtained by method 2006.03 and values obtained by the proposed microwave mixed acid digestion method or by the hot block mixed acid digestion. For Co and Mo, there was a significant difference (P = 0.05) between the AOAC 2006.03 method and the proposed microwave mixed acid method, but the differences were not highly significant (P = 0.01). Of the 28 Co and Mo values used for comparison, all but one was higher when using the proposed method extension and modification, again suggesting an enhanced recovery of some metals when using the microwave mixed acid digest. For Cr, the difference between the values obtained by 2006.03 and the values obtained by the proposed microwave mixed acid digestion were highly significant (P = 0.01), with all values higher by the proposed method modification and extension. As a result, it is likely that the proposed method extension and modification will generate higher Cr results than the parent AOAC 2006.03 method. Since the microwave mixed acid and the hot block mixed acid both consistently produced higher chromium results than AOAC 2006.03, it is more likely that AOAC method 2006.03 has a low bias with some fertilizer materials.

Comparison of Hot-Block Mixed-Acid and AOAC 2006.03 Data:

For As, Cr, Ni, and Pb, at the P = 0.05 level, there was no difference between the values obtained by method 2006.03 and values obtained by the hot block mixed acid digestion method. For Cd, Co, and Mo, there was a significant difference (P = 0.05) between 2006.03 and the hot block mixed acid method, but the differences were not highly significant (P = 0.01). Of the values reported for Cd, Co, and Mo, 63% were higher by the hot block mixed acid method, supporting the theory that mixed acids will enhance recovery of some metals. For Se, statistical comparison of the hot block mixed acid and 2006.03 data was not possible due to grossly elevated Se values generated from the hot block mixed acid data. Possibly, contamination from one of the reagents (or from the digestion vessels) resulted in these elevated levels. Since the main objective of this study was to compare the single acid AOAC 2006.03 microwave digestion with its proposed modification and extension, this matter was not pursued further.

A further representation of the enhanced recoveries using the proposed extension and modification of 2006.03 is demonstrated in Figures A4 and A5. Magruder 2006-04B was one of the materials used for the inter-laboratory digestion and method comparison study. As these figures show, with the exception of Cd, all other metal values were considerably higher when using a mixed acid digest. Given the concerns that exist regarding several of these metals, it was decided that a method modification is warranted. For all elements, enhanced recovery using the dual acid digestion system was demonstrated. For As and Cr, the recoveries doubled, and for Mo the recovery increased significantly. Overall method differences may not be highly significant (P = 0.01); however, the addition of hydrochloric acid enhances the recovery of both Group A and Group B for some fertilizer materials.

Based upon the results of the inter-laboratory study, a single laboratory validation was initiated to verify if a universal method is viable for both Group A and Group B metals in all classes of fertilizers and to evaluate if using mixed acids rather than a single acid enhances recoveries.

(1) Webb, S., Bartos, J., Boles, R., Hasty, E., Thuotte, E., & Falls, H. (2009) Simultaneous Determination of Nutritive and Nonnutritive Metals in Fertilizers by ICP-OES, September 2009, Proceedings (Abstract 1804) 123rd AOAC International Annual Meeting and Exposition, Philadelphia, PA

(2) Official Methods of Analysis, 18th Ed. (2005) AOAC International, Gaithersburg, MD Method 965.09

(3) Official Method of Analysis, 18th Ed. (2005) AOAC International, Gaithersburg, MD Method 2006.03

(4) EPA Method 3050B, Acid Digestion of Sediments, Sludges, and Soils (1996), U.S. Environmental Protection Agency, Cincinnati, OH

(5) Magruder Fertilizer Check Sample Program, www.magrudercheckssample.org (accessed on May 2013)

(6) EPA Method 3051A, Microwave Assisted Digestion of Sediments, Sludges, Soils, and Oils (2007), U.S. Environmental Protection Agency, Cincinnati, OH

Table A1. Group B results for the digestion and method comparison inter-laboratory study

Source	Sample	% Ca	% Cu	% Fe	% Mg	% Mn	% Zn
Mag 2001-08 *	2	5.17	0.006	0.23	4.54	0.161	0.148
Mag 2002-02 *	3	0.71	0.020	0.60	0.19	0.013	0.088
Mag 2002-09B *	4	2.56	1.754	24.18	0.86	4.679	6.644
Mag 2003-01 *	5	1.30	0.018	5.23	2.37	0.038	0.046
Mag 2004-10 *	6	6.52	0.120	0.43	1.77	1.555	0.454
Mag 2005-06 *	7	1.63	0.022	5.36	0.49	0.040	0.038
Mag 2006-04B *	8	6.01	0.129	1.27	3.05	13.179	4.125
Mag 2006-07 *	9	0.21	0.003	1.22	1.65	0.590	0.025
Mag 2007-03 *	10	3.17	7.252	14.62	0.38	7.274	6.079
Material A **	11	0.02	0.000	0.35	0.00	0.000	0.000
Material B **	12	11.19	0.001	1.17	2.16	0.033	0.001
Material C **	13	18.35	0.005	4.54	2.86	0.040	0.018
Material D **	14	2.31	0.008	11.03	0.21	0.063	0.018
Material E **	15	2.08	0.445	52.44	0.62	1.698	1.302

*** Magruder check sample consensus values reported for AA or atomic absorption**
****Commercial fertilizers with a range of micronutrient values determined by AOAC 965.03**

Table A2. Group A results for the digestion and method comparison inter-laboratory study

Source	Sample	As ppm	Cd ppm	Co ppm	Cr ppm	Mo ppm	Ni ppm	Pb ppm	Se ppm
Mag 2001-08*	2	5.32	2.80	8.63	**nvr**	4.96	22.08	11.87	3.20
Mag 2002-02*	3	1.10	0.89	2.92	29.32	2.26	15.44	35.06	1.67
Mag 2002-09B*	4	624.41	25.33	210.13	526.29	198.93	377.97	4302.50	**nvr**
Mag 2003-01*	5	54.19	2.72	15.36	567.65	8.19	33.75	90.74	2.86
Mag 2004-10*	6	44.89	1.55	56.86	185.12	14.33	89.67	21.64	2.47
Mag 2005-06*	7	4.47	2.79	8.10	185.07	13.95	27.80	40.42	4.92
Mag 2006-04B*	8	193.12	12.79	77.52	148.29	20.56	81.80	632.38	36.46
Mag 2006-07*	9	0.60	0.92	12.38	62.88	3.90	30.33	9.19	9.99
Mag 2007-03*	10	32.93	5.34	41.80	62.88	575.68	5342	52.15	16.13
Material A**	11	**bdl**	**bdl**	0.39	0.79	0.62	0.65	**bdl**	0.00
Material B**	12	**bdl**	**bdl**	10.33	27.05	2.58	30.33	2.72	3.80
Material C**	13	**bdl**	**bdl**	8.37	14.87	0.88	10.38	4.24	4.02
Material D**	14	**bdl**	**bdl**	11.10	31.31	0.65	18.88	**bdl**	13.35
Material E**	15	**bdl**	**bdl**	89.09	525.52	150.51	326.78	322.77	89.16

* Magruder check sample reference and reported values for the consensus method group grand average

** Commercial fertilizers with a range of concentrations as determined by AOAC 2006.03

nvr = no value reported

bdl = below instrument detection limit

Figure A1. Recovery of Fe by the various digestion methods for samples containing low-level Fe

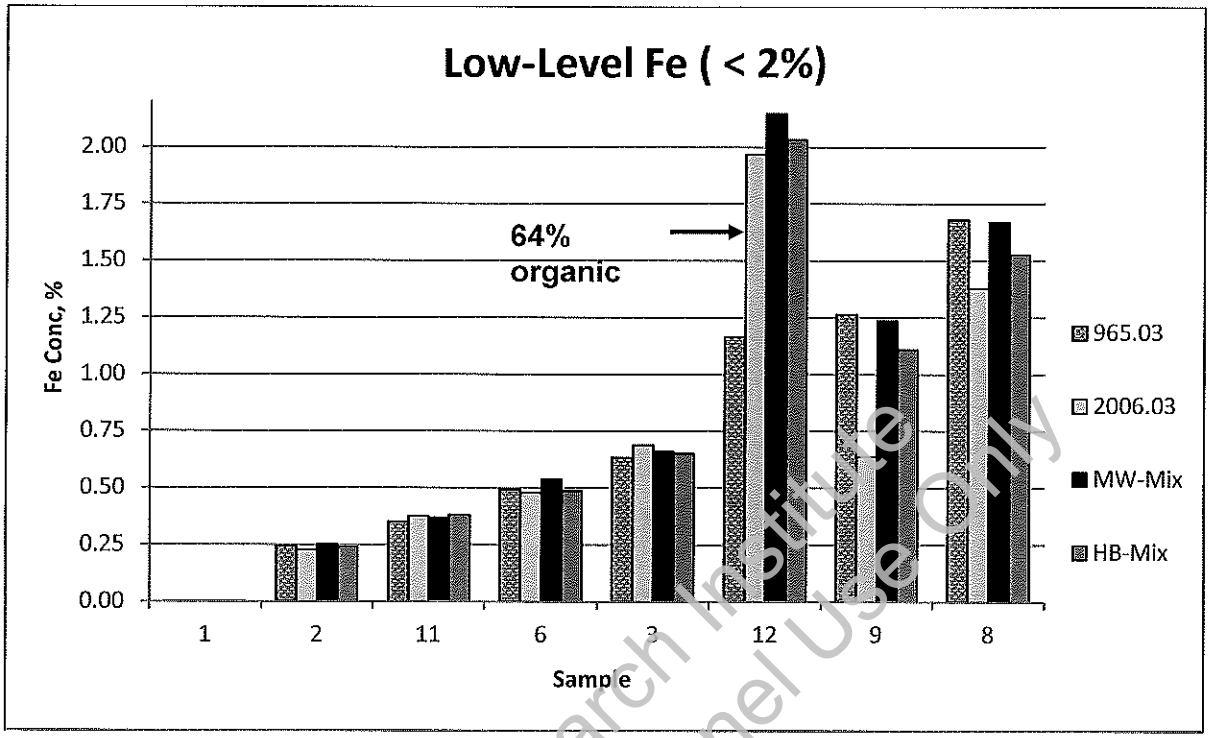


Figure A2.Recovery of Fe by the various digestion methods for samples containing mid-level Fe

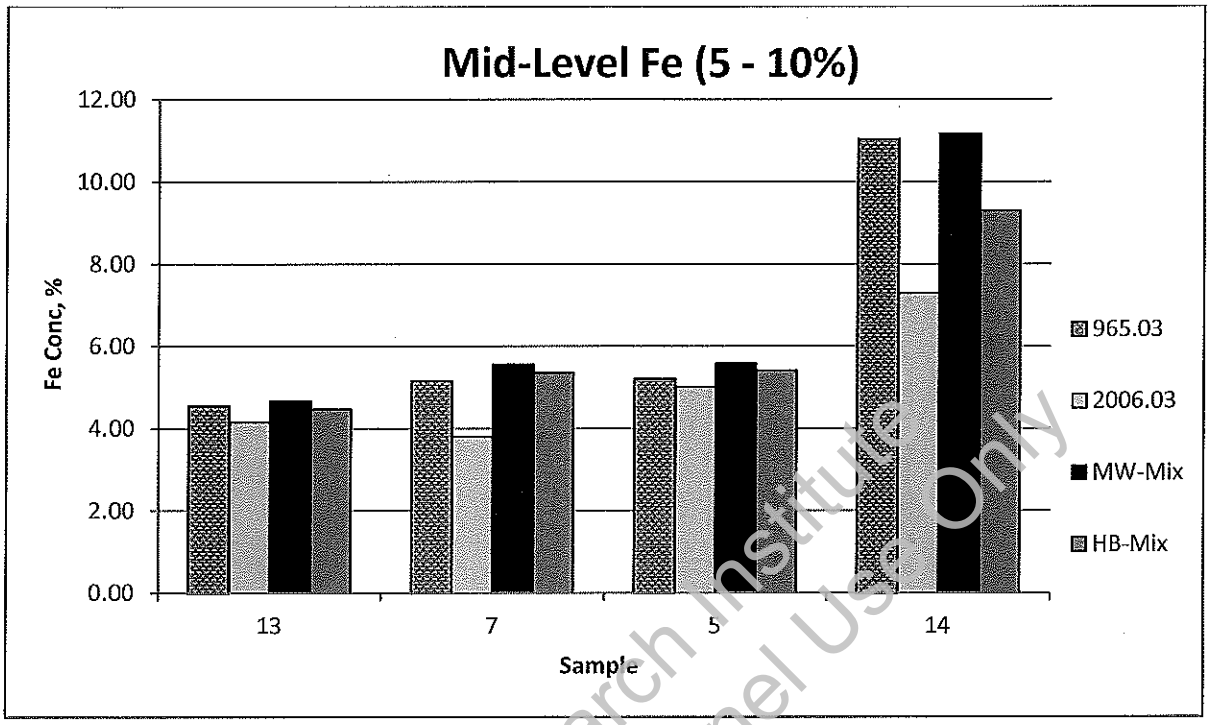


Figure A3. Recovery of Fe by the various digestion methods for samples containing high-level Fe

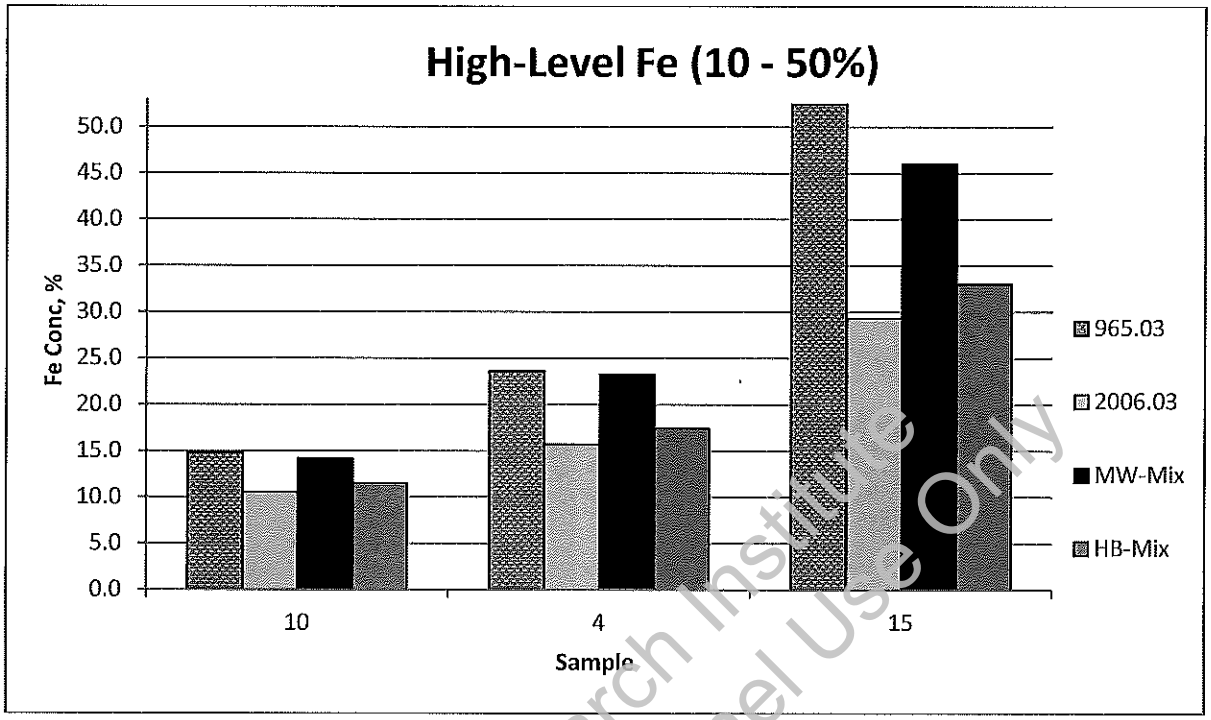


Figure A4. Results demonstrating enhanced recovery of Group A metals using the mixed acids

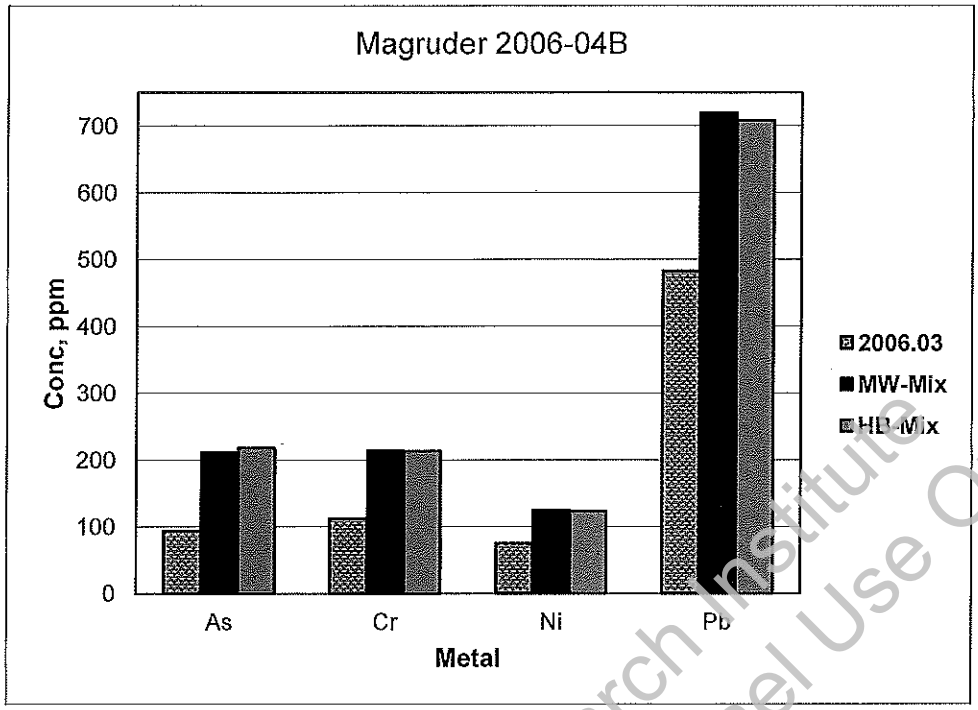
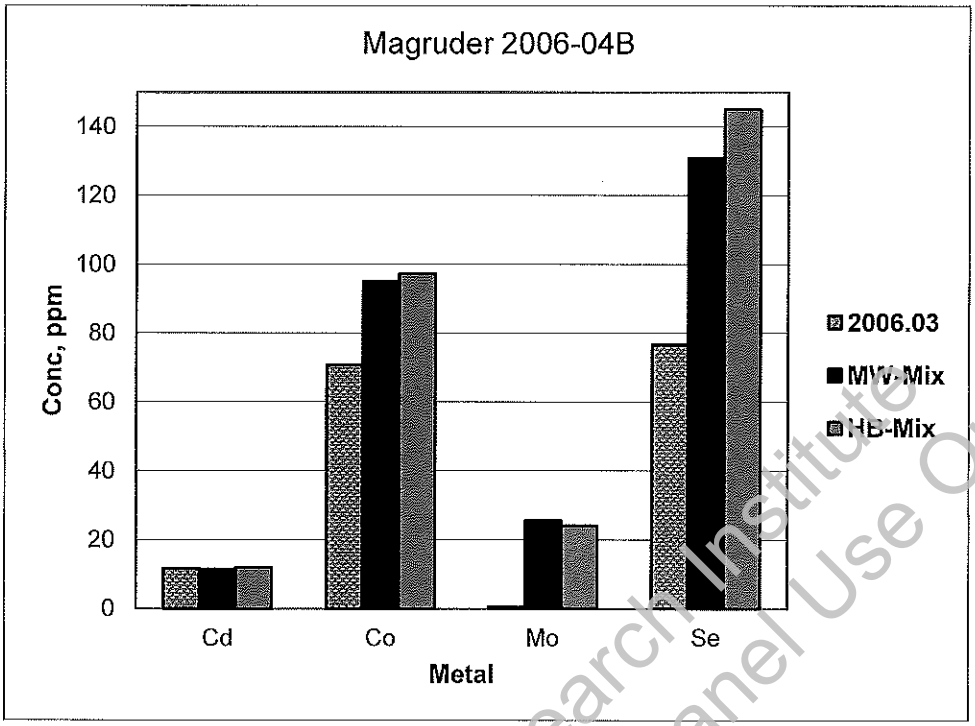


Figure A5. Results demonstrating enhanced recovery of Group A metals using the mixed acids



Division of Regulatory Services
University of Kentucky
103 Regulatory Services Bldg.
Lexington, KY 40546-0275
USA

November 1, 2014

Dear Colleague,

I have been appointed the Study Director of an AOAC collaborative study of a method for the determination of several nutrient and nonnutrient metals in fertilizers using a mixed-acid microwave digestion and analysis by ICP-OES. I am writing to formally invite your laboratory to participate in this collaborative study, as we have previously communicated. At that time you expressed an interest in participating.

The single laboratory validation method has been published in the Journal of the AOAC International Volume 97, No. 3, 2014. The collaborative study is planned for 2014 and consists of 2 phases. The first phase will involve the analysis of a set of pre-study practice samples using the method. These samples will be used to verify shipping arrangements, to allow laboratories to familiarize themselves with the method and to work out any technical issues with the method, which may arise during the familiarization timeframe. The results from the pre-study practice samples should be submitted to the study director for evaluation. If necessary, technical recommendations will be made to the individual laboratories to further refine their analysis to ensure validity of the final study results. The second phase of the study will involve the analysis of 36 study samples. This second phase will finalize the performance parameters of the nutrient and nonnutrient metals in fertilizers by microwave digestion and analysis via ICP-OES method. If the results meet the requirements of AOAC, the method will be published in the Journal of AOAC International and the Official Method of Analysis compendium.

Accompanying the study samples are several relevant documents. First is this cover letter and instructions. Second is the method SOP. Please note that it is critical to the success of the study that you follow the method exactly as written, without any modifications. If you are unable to attain the recommended chemicals, standards, supplies or instrument settings listed, please inform the Study Director prior to commencement of analyses. Also include this information in the comments section provided when reporting your results. Third is a *Data Record Sheet* on which you should record the weight of each sample and your final results. If you are missing any of these documents, or have any questions, please contact the Study Director.

Stability testing has indicated that the samples will be stable for the duration of the study period when stored at room temperature (~25° C/75° F). To address any settling and to ensure sample homogeneity, it is recommended that the sample vials be rolled on a bench top and that the liquid samples be shaken prior to taking a subsample portion.

The fertilizer samples are composed primarily of salts, so a lab coat, safety glasses and acid resistant gloves are recommended for protection. As outlined in the SOP the method requires the use of concentrated acids which should only be used in the fume hood. Allow the samples after digestion to cool for at least 30 minutes prior to uncapping and transferring the digestate to the volumetric flask and bringing to volume. Be sure to follow all safety recommendations provided in the MSDS and your laboratory chemical hygiene plan. Solutions generated from this method are considered hazardous waste due to the high acid content and should be labeled as such and disposed in the manner as proscribed by your chemical hygiene plan.

Some hard copies of the data are required for archival purposes. Record your sample weights and your final results for the practice samples (PS) and study samples (SS) on the "Data Record Sheet" (see Appendix C and D). On the Data Record Sheets, you will find a comments section where you can record any information or exceptions that will be evaluated by the Study Director. While an electronic or hard copy of the data does not need to be reported to the Study Director, it is recommended that a hard copy of the data be archived for seven years. If this is impractical, you may print a copy of your ICP-OES sample results, which can be archived by the Study Director.

Please report your practice study results by January 15, 2015 and your study sample results by December 31, 2015 to Sharon Webb.

Your contribution to this collaborative study is highly valued and appreciated. Should you have any questions or concerns, please contact the Study Director.

Respectfully,

Sharon F. Webb, Ph.D.
Study Director
University of Kentucky
Division of Regulatory Services
Email: sharon.webb@uky.edu
Phone: 859-218-2451

Practice Sample Data Record Sheet - ICP for Nonnutrients in Fertilizers Collaborative Study

Lab Name:	
Contact Person:	
Reporting Date:	

Data for First Analysis

Sample ID	Sample Wt, g	As, ppm	Cd, ppm	Co, ppm	Cr, ppm	Mo, ppm	Ni, ppm	Pb, ppm	Se, ppm
1PS									
2PS									
3PS									
4PS									
5PS									
6PS									
7PS									
8PS									
9PS									
10PS									

Data for Second Analysis

Sample ID	Sample Wt, g	As, ppm	Cd, ppm	Co, ppm	Cr, ppm	Mo, ppm	Ni, ppm	Pb, ppm	Se, ppm
1PS									
2PS									
3PS									
4PS									
5PS									
6PS									
7PS									
8PS									
9PS									
10PS									

If you have any questions about reporting, please contact Sharon Webb (sharon.webb@uky.edu; phone: 859-218-2451)

Comments:
(Optional)

Practice Sample Data Record Sheet - ICP for Nutrients in Fertilizers Collaborative Study

Lab Name:	
Contact Person:	
Reporting Date:	

Data for First Analysis							
Sample ID	Sample Wt, g	Ca, %	Cu,ppm	Fe, %	Mg, %	Mn, %	Zn, %
1PS							
2PS							
3PS							
4PS							
5PS							
6PS							
7PS							
8PS							
9PS							
10PS							

Data for Second Analysis							
Sample ID	Sample Wt, g	Ca, %	Cu,ppm	Fe, %	Mg, %	Mn, %	Zn, %
1PS							
2PS							
3PS							
4PS							
5PS							
6PS							
7PS							
8PS							
9PS							
10PS							

If you have any questions about reporting, please contact Sharon Webb (sharon.webb@uky.edu;
phone: 859-218-2451)

Comments:
(Optional)

Sample Data Record Sheet - ICP for Nonnutrients in Fertilizers Collaborative Study

Lab Name:
Contact Person:
Reporting Date:

Sample ID	Sample Wt, g	As, ppm	Cd, ppm	Co, ppm	Cr, ppm	Mo, ppm	Ni, ppm	Pb, ppm	Se, ppm
1SS									
2SS									
3SS									
4SS									
5SS									
6SS									
7SS									
8SS									
9SS									
10SS									
11SS									
12SS									
13SS									
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22SS									
23SS									
24SS									
25SS									
26SS									
27SS									
28SS									
29SS									
30SS									

If you have any questions about reporting, please contact Sharon Webb (sharon.webb@uky.edu; phone: 859-218-2451)

Comments:
(Optional)

Sample Data Record Sheet - ICP for Nutrients in Fertilizers Collaborative Study

Lab Name:	
Contact Person:	
Reporting Date:	

Sample ID	Sample Wt, g	Ca, %	Cu,ppm	Fe, %	Mg, %	Mn, %	Zn, %
1SS							
2SS							
3SS							
4SS							
5SS							
6SS							
7SS							
8SS							
9SS							
10SS							
11SS							
12SS							
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25SS							
26SS							
27SS							
28SS							
29SS							
30SS							

If you have any questions about reporting, please contact Sharon Webb (sharon.webb@uky.edu; phone: 859-218-2451)

Comments:
(Optional)

SAFETY CHECKLIST

(To be completed by the Study Director)

Method Title: Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganese, Molybdenum, Nickel, Selenium and Zinc in Fertilizers by Microwave Acid Digestion and ICP-OES Detection: Single Laboratory Validation of a Modification and Extension of AOAC 2006.03.

Please click on desired check box:

QUESTIONS	YES	NO
1. Are any materials used or compounds formed that are explosive or flammable?	☐	X
2. Are there any side reactions that could occur that might produce flammable or explosive products or conditions?	☐	X
3. Are there any hazards created from electric or mechanical equipment?	X	☐
4. Are pressure differentials created that could result in an explosion or implosion?	X	
5. Are any substances used or formed which are:		
a) radioactive?	☐	X
b) carcinogenic?	☐	X
c) mutagenic?	☐	X
d) tetratogenic?	☐	X
e) abortogenic:	☐	X
f) otherwise a significant health hazard?	☐	X
6. Would there be increased hazards if the reaction temperature were increased even modestly?	☒	
7. Are special procedures required if a spill of the reaction mixture occurs?	☐	X
8. Is there a risk in producing a dangerous aerosol?	☐	X
9. Are special procedures required for the disposal of reagents or reaction products? reaction products?	☒	
10. Are there any organisms and/or their products used/present that are:		
a) pathogenic?	☐	X
b) allergenic?	☐	X
c) carcinogenic?	☐	X
d) mutagenic?	☐	X
e) tetratogenic?	☐	X
f) otherwise a significant health hazard?	☐	X
11. Are there any potential hazards in handling or storage of reagents, test samples, or standards?	X	☐

	YES	NO
12. Are there any other hazards that should be addressed regarding the method?	☐	X
13. Does your method use chlorinated solvents?	☐	X
14. If "yes" to question13, have non-chlorinated solvents equivalent to chlorinated solvents been investigated?	n/a	

If the answer to any question above is yes:

1. Include appropriate precautionary statements in method write-up.

2. Provide specific information on hazard and attach it to this sheet for review by the Safety Committee Advisor.

Comments: Concentrated Hydrochloric Acid and concentrated Nitric Acid are used as digestion solvents and to make up of the standards used in this procedure. Preparation of these is limited to fume hoods. Care must be taken when adding the acids to the samples because some fertilizer materials upon contact with acids may produce an exothermic reaction. Once the samples are digested by the microwave, a sufficient time must be allowed before uncapping and transferring to the volumetric flasks. Most commercial microwave digestion units are equipped with temperature and/or pressure monitors and other safety devices such as venting and resealing vessels and containment systems. Proper safety procedures are listed in the microwave user's manual and should always be followed.

Name: **Dr. Sharon F. Webb**
Study Director

Date: **06/13/14**

Please complete, date and email to Sharon.webb@uky.edu .

2.6.35

AOAC Official Method 2006.03
Arsenic, Cadmium, Cobalt, Chromium, Lead, Molybdenum, Nickel, and Selenium in Fertilizers

Microwave Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry

First Action 2006
Final Action 2009

[Applicable to analysis of As, Cd, Co, Cr, Pb, Mo, Ni, and Se in all classes of fertilizers. Limit of quantitation (LOQ; mg/kg): As, 14.4; Cd, 2.46; Co, 3.3; Cr, 33.9; Mo, 7.5; Ni, 8.1; Pb, 13.2; Se 13.2.]

See Tables **2006.03A–H** for the results of the interlaboratory study supporting acceptance of the method. Note that materials with iron content >5% require special cautions as noted in the method, and may experience varying degrees of degradation of precision.

Digestion

A. Principle

Test portion is heated with nitric acid in closed vessel microwave digestion system at 200 C.

B. Apparatus

Microwave.—Commercial microwave designed for laboratory use at 200 C, with closed vessel system and controlled temperature ramping capability. It is recommended that vessel design be selected that will withstand the maximum possible pressure, since some organic fertilizer products, and also carbonates if not given sufficient time to predigest, will generate significant pressure during digestion. (Vessels can reach 700 psi or more on occasion.) Vent according to manufacturer's recommendation. (*Caution*: Microwave operation involves hot pressurized acid solutions. Use appropriate face protection and laboratory clothing.)

C. Reagents

(**a**) *Water.*—Use 18 Megaohm water throughout for dilution.

(**b**) *Concentrated HNO_3.*—Use trace metal grade HNO_3 throughout.

D. Determination

(*Caution*: Observe standard precautions with concentrated acid. When dispensing acid or venting vessels, use gloves, face protection, and laboratory coats. Never remove hot vessels from microwave; wait until they are near room temperature. Keep microwave door closed while vessels are hot. The door is the primary safety device if a vessel vents.)

Prepare solid samples as in 929.02 (*see* 2.1.05). Accurately weigh 1.0000 ± 0.010 g (0.5000 g for organic matrixes) test portion to digestion vessel. Use weighing paper insert to line the vessel walls during sample transfer, to keep sample from adhering to sides of vessel. Fluid samples may be weighed directly after mixing. Add 10.0 ± 0.2 mL trace metal grade HNO_3, loosely cap vessels without sealing, predigest at room temperature until vigorous foaming subsides, or overnight if time allows. Seal vessels according to manufacturer's directions and place in microwave. With power setting appropriate to microwave model and number of vessels used, ramp temperature from ambient to 200 C in 15 min. Hold at 200 C for 20 min. Cool vessels according to manufacturer's directions, vent, and transfer digests to 100 mL volumetric flasks, dilute to volume, and mix. Transfer to polypropylene containers within 2 h, unless solutions are to be analyzed immediately. Dilute samples that are found to be above the standard curve range, or have content of metals higher than 1000 mg/kg. Final dilutions require addition of appropriate amounts of HNO_3 to maintain the proportion of 10% HNO_3 in the final solution to be analyzed.

Detection

E. Principle

Digested test solution, or an appropriate dilution, is presented to the inductively coupled plasma-optical emission spectrometry (ICP-OES) instrument calibrated with acid matched standard calibrant solutions. An ionization buffer (cesium) is used to minimize easily ionizable element (EIE) effects, and scandium and/or beryllium are used as internal standard(s).

F. Instrumentation

(**a**) *ICP emission spectrometer.*—Capable of determining multiple wavelengths for each element of interest. A 3-channel peristaltic pump is desirable to avoid the necessity of having to manually add ionization buffer and internal standard to each sample solution. Use a Meinhard or Seaspray nebulizer and Cyclonic Spray Chamber, or other components designed to optimize aerosol and maximize precision. Select sample and internal standard pump tubes, and peristaltic pump rotation speed, with regard to manufacturer's recommendations, but try to keep sample and internal standard pump tubes of similar size, to maximize mixing accuracy, while maintaining needed detection levels.

The analyst must compensate for EIE effects in the plasma since fertilizer materials can contain substantial concentrations of elements that provide a significant source of electrons to the plasma, such as K and Ca. The presence of ionization buffer in all samples and standards will minimize the effect of varying concentrations of EIEs in the sample. Power settings and nebulizer gas flow should be optimized for robust plasma conditions. The analyst needs to ensure that the Mg 285.213:Mg 280.271 ratio (Mermet principle of robust plasma) demonstrates robust operating conditions in accordance with the ratio established by the instrument manufacturer. Two or 3 replicate readings of the same sample are desirable, with relatively longer integration time to minimize noise. Properly optimized instruments should have internal standard ratios for most samples consistently in the range 0.9 to 1.0. It is unusual to have the ratio lower than 0.8 over a very wide range of fertilizer material types. The occurrence of lower ratios is cause for troubleshooting. Select ionization buffer/internal standard solution, **G**(**d**), such that after mixing sample and internal standard solutions using the instrument's peristaltic pump, the combined solution presented to the nebulizer contains 2200 mg/kg or greater cesium chloride; 0.75 to 1.0 mg/kg internal standard; and 7.2 mg/mL or less actual fertilizer material. (For example, these conditions would be met with a 1 g sample digested and diluted to 100 mL before instrument analysis; an ionization buffer/internal standard solution of 8000 mg/kg cesium chloride and 3 mg/kg scandium and/or beryllium internal standard(s); and pump tubes of white/white (1.02 mm id) sample and orange/white (0.64 mm id) internal standard, the white/white contributing about 72%, and the orange/white contributing about 28%, to the final nebulized solution.)

At a minimum, all sample instrument responses for each element should be corrected using one internal standard wavelength. However, best practice is to utilize similar transitions between analyte and internal standard. For example, the As 188.980 wavelength is from arsenic in the atomic state, so the internal

standard wavelength used for correction should also be from the atomic state, such as Sc 361.383. Conversely, match ionic sample lines with ionic internal standard lines. (*Note*: Do not use yttrium as an internal standard, since it is found native at low levels in some phosphate ore sources.)

(**b**) *ICP wavelengths.*—A number of wavelengths may be used for analysis of the 8 elements of interest, depending on the capability of the analytical instrument used. As a minimum, select at least 2 wavelengths for each element of interest, and report the average value of closely agreeing results, except for lead and selenium, for which there is only one reliable wavelength available. Following is a list of suggested wavelengths, not in any priority order, that have been found acceptable for most fertilizer materials. Other lines of appropriate sensitivity, free of interferences or corrected for interferences, may be just as acceptable. However, it is imperative that instrument response (both instrument graphic output and calculated concentration) be reviewed for each sample and element. Fertilizer materials are extremely variable in composition, and a wide concentration range of potential interfering elements is expected, so no single wavelength will work in every instance. Occasional data with interference will inevitably be found, and must be eliminated from inclusion in the mean calculation for that particular element and sample.

Wavelengths (nm): As: 188.980, 193.696; Cd: 214.439, 226.502; Co: 228.615, 230.786, 235.341; Cr: 205.560, 267.716, 276.653; Mo: 201.512, 202.032, 203.846, 204.598; Ni: 216.555, 222.295, 222.486, 227.877, 231.604, 239.452; Pb: 220.353; Se: 196.026; Sc: 361.383, 431.408; Be: 234.861, 249.473.

(**c**) *Wavelength interference treatment.*—Interelement interference can cause substantial error in analytical results. Error can be minimized by several techniques: (*1*) Three or more analytical lines may be used for a given element, and when an interferent is present in a particular line, the result for that line is omitted from the mean value reported. (*2*) Certain vendors' instrument software has the capability of mathematically modeling potential interferents, and deconvoluting the instrument response into an analytical element portion and an interferent portion. (*3*) Interelement correction is an alternative mathematical technique to use with instruments for which mathematic modeling is not available, or where direct spectral overlap negates use of the deconvolution technique.

The following lines, if used, must utilize one of the correction techniques; corrections for other lines may be applied as needed and appropriate: (*1*) As 188.980: Correct for Cr interference at 188.995, or verify that Cr is not present in the test portion analyzed. (*2*) As 193.696: Fe affects the arsenic peak. Remove with an Fe model, or verify that Fe is not present in the test portion analyzed. (*3*) Cd 214.439 and 226.502: Fe, present in many fertilizers, interferes with both suggested Cd wavelengths. Mathematically correct instrument Cd response for the interference, or verify analytically that Fe is not present in the test portion analyzed. (*4*) Pb 220.353: Mathematically correct instrument Pb response for Fe interference, or verify that Fe is not present in the test portion analyzed. (*5*) Se 196.026: Mathematically correct instrument Se response for Fe interference, or verify that Fe is not present in the test portion analyzed.

(**d**) *ICP instrument calibration*.—Prepare working standard solutions from commercial stock standards at 1000 mg/kg. Custom blended multielement stock standard in HNO_3 is acceptable. Prepare a minimum of 5 working standards at 0.1, 0.5, 1.0, 5.0, and 10.0 mg/kg, plus blank, of each element, in 10% trace metal grade HNO_3. Working standards should be in the linear range, with correlation coefficients of at least 0.9999.

G. Reagents

(**a**) *Water*.—Use 18 Megaohm water for dilution.

(**b**) *HNO_3.*—Use trace metal grade HNO_3.

(**c**) *0.5% Triton X100 solution*.—Dilute 0.5 mL Triton X100 to 100 mL with H_2O.

(**d**) *Ionization buffer/internal standard solution.*—Weigh 8.0 g CsCl into a 1000 mL acid-washed volumetric flask. Add 3 mL each of ICP grade scandium and beryllium 1000 mg/kg stock solution, as internal standards. Also add 1 mL of 0.5% Triton X100, dilute to volume, and mix. Store in a polypropylene bottle. (*Note*: Reagent concentrations assume the use of white/white, 1.02 mm id sample pump tube, and orange/white, 0.64 mm id internal standard pump tube. If the sample and internal standard solutions are mixed in different proportions by the instrument's peristaltic pump, then adjust the reagent concentrations to meet concentration requirements of mixed solution nebulized by the instrument, as outlined in **F**. Note that sample and internal standard solution mixing ratio is proportional to pump tube flow rates, not proportional to pump tube IDs.)

(**e**) *Stock standard solution.*—Working standards can be prepared from ICP grade individual element 1000 mg/kg commercial stock standard solutions. However, it is also acceptable to use commercially prepared custom blended stock standard mixtures containing all of the 8 elements at 1000 mg/kg. A number of companies provide this stock standard service.

(**f**) *10 mg/kg intermediate stock standard solution for preparation of low-level working standards.*—Dilute 5.0 mL of stock standard solution to 500 mL. Prepare fresh each time standards are prepared, and use immediately after preparation.

(**g**) *Working standard solutions.*—Standards are designed to have the same acid concentration as digested test solutions. Date all calibration solutions when made, which should be stable for at least 1 month, but not longer than 2 months. Monitor standard curve fit and intensity for signs of change and degradation over time. (*1*) *10 mg/kg elements*.—Pipet 5.0 mL of combined 1000 mg/kg element stock solution into a 500 mL acid-washed volumetric flask. Add 50 mL of trace metal grade HNO_3, dilute to volume with H_2O, mix, and transfer to acid-washed polypropylene bottle. (*2*) *5 mg/kg elements*.—Pipet 5.0 mL of combined 1000 mg/kg element stock solution into a 1000 mL acid-washed volumetric flask. Add 100 mL of trace metal grade HNO_3, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle. (*3*) *1 mg/kg elements*.—Pipet 50.0 mL of 10 mg/kg intermediate stock solution into a 500 mL acid-washed volumetric flask. Add 50 mL of trace metal grade HNO_3, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle. (*4*) *0.5 mg/kg elements*.—Pipet 25.0 mL of 10 mg/kg intermediate stock solution into a 500 mL acid-washed volumetric flask. Add 50 mL of trace metal grade HNO_3, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle. (*5*) *0.1 mg/kg elements*.—Pipet 5.0 mL of 10 mg/kg intermediate stock solution into a 500 mL acid-washed volumetric flask. Add 50 mL of trace metal grade HNO_3, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle. (*6*) *0.0 mg/kg elements (blank)*.—Add 50 mL of trace metal grade HNO_3 into a 500 mL

Table 2006.03A. Blind duplicate statistics for arsenic

Sample ID	Sample description	$\bar{X}$, mg/kg	s_r[a]	s_R[b]	RSD_r[c], %	RSD_R[d], %	r[e]	R[f]	HorRat	Outliers
4321/2025	Metal Fe oxysulfate	22.15	9.51	19.26	42.93	86.98	26.63	53.95	8.67	0/0
4031/5938	Magruder 2002-09B	263.18	96.95	126.22	26.58	47.96	195.88	353.43	6.94	0/0
6341/1436	31% $ZnSO_4$	0.47	0.72	1.21	154.18	257.52	2.03	3.38	14.37	0/0
5488/5890	Zinc oxysulfate	4.87	0.69	3.07	14.21	63.12	1.94	8.60	5.01	0/0
2818/7669	Granulated mine waste	4945.31	301.32	1034.06	6.09	20.91	843.70	2895.37	4.70	1/2
1615/2056	Metal oxysulfate	2432.25	256.97	570.69	10.56	23.46	719.50	1597.94	4.74	0/2
9978/4085	Florida DAP	11.00	1.18	3.96	10.77	35.98	3.32	11.08	3.23	0/2
3313/6267	Western MAP	9.75	4.07	5.74	41.73	58.87	11.39	16.07	5.18	0/0
7161/6990	Western DAP	15.77	0.88	4.11	5.61	26.03	2.48	11.49	2.46	1/0
7999/3375	DAP from North African rock	22.43	2.10	4.26	9.36	18.99	5.88	11.92	1.90	0/0
6501/4812	NC MAP	2.36	0.42	1.89	17.60	79.97	1.17	5.29	5.69	0/2
7738/7418	China DAP	13.04	1.73	3.81	13.27	29.26	4.84	10.67	2.69	0/0
3917/8165	Magruder 2003-11	185.15	7.93	18.35	4.27	9.90	22.19	51.39	1.36	0/0
4459/8931	Magruder 2004-07	175.28	13.74	19.97	7.84	11.39	38.48	55.91	1.55	0/1
6895/4337	N-P-K blend + iron	6.89	0.72	1.27	10.45	18.50	2.02	3.57	1.55	0/2
7946/3692	Magruder 2001-03	7.44	1.21	2.72	16.27	36.60	3.39	7.63	3.10	0/2
8873/9469	N-P-K lawn product blend	–0.33	0.05	1.44	–14.38	–441.57	0.13	4.03		2/0
9886/9774	Organic biosolid	7.35	3.91	5.30	53.17	72.15	10.94	14.34	6.09	2/0
4626/8088	Organic mixed fertilizer + biosolid	12.74	2.01	7.22	15.74	56.70	5.61	20.22	5.20	0/0
6411/3401	Composted manure	4.16	1.88	2.70	45.14	64.87	5.25	7.55	5.02	0/1
3716/4606	Fe humate	47.83	9.67	18.25	20.22	38.15	27.08	51.09	4.27	0/0
4397/5149	Bone meal	2.80	0.76	2.40	27.32	85.92	2.14	6.73	6.27	1/1
4760/2264	Blood/feather/bone	2.91	2.01	2.59	68.89	88.77	5.62	7.24	6.52	0/2
6883/6583	Feather/bone/sulfate/gypsum	1.85	0.46	2.79	25.03	150.70	1.30	7.80	10.33	0/3
4924/7924	NIST SRM 694 Western Phos Rock	11.95	0.81	7.90	6.75	66.06	2.26	22.11	6.00	0/2
4131/1340	AFPC rock check 22	10.569	1.93	4.34	18.24	41.10	5.39	12.16	3.66	1/0
5682/8537	Peru rock	14.04	0.87	2.94	6.17	20.91	2.43	8.22	1.95	1/0
7883/3887	African rock	13.27	0.72	4.97	5.44	37.42	2.02	13.91	3.45	1/0

[a] s_r = Repeatability standard deviation.

[b] s_R = Reproducibility standard deviation.

[c] RSD_r = Repeatability relative standard deviation.

[d] RSD_R = Reproducibility relative standard deviation.

[e] r = Repeatability value.

[f] R = Reproduciblity value.

Table 2006.03B. Blind duplicate statistics for cadmium

Sample ID	Sample description	$\bar{X}$, mg/kg	s_r	s_R	RSD_r, %	RSD_R, %	r	R	HorRat	Outliers
4321/2025	Metal Fe oxysulfate	2.25	0.88	3.19	39.14	141.86	2.46	8.93	10.02	1/0
4031/5938	Magruder 2002-09B	7.56	1.49	2.37	19.65	31.30	4.16	6.62	2.65	0/2
6341/1436	31% $ZnSO_4$	0.94	0.44	0.44	46.56	46.56	1.23	1.23	2.88	0/0
5488/5890	Zinc oxysulfate	21.28	0.13	6.10	0.61	28.67	0.36	17.08	2.84	2/0
2818/7669	Granulated mine waste	36.64	1.17	3.33	3.20	9.09	3.28	9.33	0.98	1/0
1615/2056	Metal oxysulfate	22.58	0.96	2.38	4.25	10.52	2.68	6.66	1.05	1/0
9978/4085	Florida DAP	14.94	3.48	4.80	23.33	32.14	9.76	13.44	3.02	1/0
3313/6267	Western MAP	214.59	6.57	16.89	3.06	7.87	18.39	47.29	1.10	0/0
7161/6990	Western DAP	85.28	0.44	6.96	0.51	8.16	1.22	19.48	1.00	1/0
7999/3375	DAP from North African rock	26.69	1.55	2.50	5.79	9.37	4.33	7.00	0.96	0/0
6501/4812	NC MAP	55.29	0.69	6.44	1.25	12.02	1.94	18.60	1.37	1/0
7738/7418	China DAP	0.52	0.03	0.39	5.13	75.83	0.07	1.10	4.30	1/0
3917/8165	Magruder 2003-11	15.52	0.43	1.77	2.77	11.42	1.20	4.96	1.08	0/0
4459/8931	Magruder 2004-07	64.04	1.89	3.80	2.95	5.93	5.29	10.63	0.69	0/0
6895/4337	N-P-K blend + iron	33.17	1.40	2.41	4.23	7.28	3.93	6.76	0.77	0/0
7946/3692	Magruder 2001-03	42.81	1.07	2.84	2.50	6.63	2.99	7.94	0.73	0/0
8873/9469	N-P-K lawn product blend	0.40	0.02	0.32	4.21	80.66	0.05	0.90	4.39	1/1
9886/9774	Organic biosolid	4.19	0.13	1.45	3.18	34.65	0.37	4.06	2.69	0/0
4626/8088	Organic mixed fertilizer + biosolid	0.54	0.10	1.13	17.85	209.26	0.27	3.17	11.93	2/0
6411/3401	Composted manure	0.12	0.03	0.53	25.30	439.21	0.09	1.49	19.99	1/0
3716/4606	Fe humate	0.57	0.38	2.45	66.74	430.46	1.06	6.87	24.72	1/0
4397/5149	Bone meal	2.97	0.35	0.77	11.80	25.76	0.98	2.14	1.90	0/0
4760/2264	Blood/feather/bone	0.73	0.47	0.75	64.63	102.90	1.33	2.09	6.08	0/0
6883/6583	Feather/bone/sulfate/gypsum	0.54	0.14	0.51	25.84	94.72	0.39	1.43	5.40	0/0
4924/7924	NIST SRM 694 Western Phos Rock	128.75	4.55	14.27	3.53	11.08	12.74	39.95	1.44	0/0
4131/1340	AFPC rock check 22	5.04	1.14	1.50	22.67	29.68	3.20	4.19	2.37	0/0
5682/8537	Peru rock	12.94	0.79	1.33	6.08	10.28	2.20	3.73	0.95	0/0
7883/3887	African rock	36.94	0.60	3.52	1.63	9.54	1.68	9.87	1.03	1/0

Table 2006.03C. Blind duplicate statistics for cobalt

Sample ID	Sample description	$\bar{X}$, mg/kg	s_r	s_R	RSD_r, %	RSD_R, %	r	R	HorRat	Outliers
4321/2025	Metal Fe oxysulfate	97.75	4.03	6.50	4.12	6.65	11.28	18.19	0.83	0/0
4031/5938	Magruder 2002-09B	195.61	18.27	33.56	9.34	17.16	51.16	93.97	2.37	0/0
6341/1436	31% $ZnSO_4$	4.00	0.47	0.75	11.75	18.76	1.32	2.10	1.44	0/0
5488/5890	Zinc oxysulfate	4.79	0.25	1.97	5.16	41.06	0.69	5.51	3.25	1/0
2818/7669	Granulated mine waste	17.33	0.69	2.50	4.00	14.41	1.94	6.99	1.38	0/0
1615/2056	Metal oxysulfate	23.01	0.81	2.30	3.53	9.98	2.27	6.43	1.00	0/2
9978/4085	Florida DAP	2.64	0.27	1.04	10.27	39.49	0.76	2.92	2.86	0/0
3313/6267	Western MAP	8.91	0.26	1.16	2.94	13.06	0.73	3.26	1.13	1/0
7161/6990	Western DAP	4.05	0.05	0.66	1.31	16.26	0.15	1.84	1.25	1/0
7999/3375	DAP from North African rock	1.24	0.23	0.62	18.49	49.60	0.64	1.73	3.20	0/0
6501/4812	NC MAP	2.35	0.15	1.23	6.40	52.54	0.42	3.46	3.73	0/0
7738/7418	China DAP	1.16	0.03	0.62	2.39	53.33	0.08	1.73	3.41	1/0
3917/8165	Magruder 2003-11	45.20	3.85	13.39	8.52	29.62	10.79	37.49	3.29	0/0
4459/8931	Magruder 2004-07	532.78	13.07	34.93	2.45	6.56	36.60	97.82	1.05	0/0
6895/4337	N-P-K blend + iron	6.73	0.50	0.96	7.43	14.28	1.40	2.69	1.19	0/0
7946/3692	Magruder 2001-03	6.22	0.58	1.19	9.28	19.12	1.62	3.33	1.57	0/0
8873/9469	N-P-K lawn product blend	4.34	0.70	1.25	16.02	28.79	1.95	3.50	2.25	0/0
9886/9774	Organic biosolid	21.25	1.06	2.60	4.98	12.26	2.96	7.29	1.21	1/0
4626/8088	Organic mixed fertilizer + biosolid	5.61	0.45	4.01	8.07	71.61	1.27	11.24	5.80	0/0
6411/3401	Composted manure	1.63	0.44	0.89	27.21	54.67	1.24	2.50	3.68	0/0
3716/4606	Fe humate	10.67	0.98	5.24	9.14	49.08	2.73	14.67	4.38	1/0
4397/5149	Bone meal	0.90	0.09	0.79	9.63	87.55	0.24	2.21	5.39	1/0
4760/2264	Blood/feather/bone	3.54	0.36	0.84	10.24	23.64	1.01	2.34	1.79	1/0
6883/6583	Feather/bone/sulfate/gypsum	0.88	0.10	0.84	10.80	95.05	0.27	2.35	5.83	0/0
4924/7924	NIST SRM 694 Western Phos Rock	2.381	0.10	0.68	4.32	28.59	0.29	1.90	2.04	1/0
4131/1340	AFPC rock check 22	3.69	0.13	0.58	357	15.63	0.37	1.61	1.19	1/0
5682/8537	Peru rock	3.73	0.13	0.57	3.43	15.24	0.36	1.59	1.16	2/0
7883/3887	African rock	1.56	0.23	0.76	14.54	48.56	0.63	2.11	3.24	0/0

Table 2006.03D. Blind duplicate statistics for chromium

Sample ID	Sample description	$\bar{X}$, mg/kg	s_r	s_R	RSD_r, %	RSD_R, %	r	R	HorRat	Outliers
4321/2025	Metal Fe oxysulfate	731.45	73.77	167.68	10.09	22.92	206.57	469.51	3.87	0/0
4031/5938	Magruder 2002-09B	396.99	56.39	78.37	14.20	19.74	157.90	219.44	3.04	0/0
6341/1436	31% $ZnSO_4$	1.88	0.46	1.14	24.30	60.69	1.28	3.20	4.17	2/0
5488/5890	Zinc oxysulfate	159.52	2.09	10.22	1.31	6.41	5.84	28.62	0.86	1/0
2818/7669	Granulated mine waste	38.25	1.40	8.68	3.66	22.70	3.92	24.31	2.46	1/0
1615/2056	Metal oxysulfate	101.15	5.65	19.82	5.59	19.59	15.83	55.50	2.45	0/0
9978/4085	Florida DAP	74.40	0.41	3.11	0.55	4.18	1.14	8.71	0.50	2/0
3313/6267	Western MAP	566.16	3.84	29.14	8	5.15	10.74	81.58	0.84	1/0
7161/6990	Western DAP	393.37	3.94	14.31	1.00	3.64	11.04	40.08	0.56	1/0
7999/3375	DAP from North African rock	281.91	8.12	18.76	2.88	6.66	22.74	52.54	0.97	0/0
6501/4812	NC MAP	341.28	10.40	29.96	3.05	8.78	29.12	83.89	1.32	0/1
7738/7418	China DAP	18.11	0.23	2.25	1.26	12.43	0.64	6.30	1.20	1/0
3917/8165	Magruder 2003-11	164.40	17.07	21.00	10.38	12.77	47.80	58.79	1.72	0/1
4459/8931	Magruder 2004-07	169.49	4.68	16.01	2.76	9.44	13.10	44.82	1.28	2/0
6895/4337	N-P-K blend + iron	276.54	10.46	29.49	3.78	10.66	29.30	82.57	1.55	1/0
7946/3692	Magruder 2001-03	196.91	2.12	9.23	1.08	4.69	5.95	25.85	0.65	1/0
8873/9469	N-P-K lawn product blend	5.84	0.32	1.84	5.52	31.55	0.90	5.16	2.57	0/3
9886/9774	Organic biosolid	115.55	3.11	5.07	2.69	4.39	8.70	14.21	0.56	1/0
4626/8088	Organic mixed fertilizer + biosolid	5980.93	59.18	265.44	0.99	4.44	165.69	743.23	1.03	1/0
6411/3401	Composted manure	108.85	7.35	11.43	6.75	10.50	20.58	32.00	1.33	0/0
3716/4606	Fe humate	134.77	9.54	23.18	7.08	17.20	26.70	64.89	2.25	0/0
4397/5149	Bone meal	3.64	0.37	1.76	10.04	48.25	1.02	4.92	3.66	0/0
4760/2264	Blood/feather/bone	8.64	1.66	1.66	19.19	19.19	4.64	4.64	1.66	0/0
6883/6583	Feather/bone/sulfate/gypsum	18.01	0.92	2.70	5.12	14.99	2.58	7.56	1.45	0/0
4924/7924	NIST SRM 694 Western Phos Rock	746.72	20.65	88.62	2.77	11.87	57.83	248.13	2.01	0/0
4131/1340	AFPC rock check 22	51.01	0.45	4.27	0.87	8.37	1.25	11.96	0.95	1/0
5682/8537	Peru rock	119.24	3.70	10.11	3.10	8.48	10.36	28.31	1.09	0/0
7883/3887	African rock	109.95	2.49	11.30	2.26	10.28	6.97	31.65	1.30	0/0

Table 2006.03E. Blind duplicate statistics for molybdenum

Sample ID	Sample description	$\bar{X}$, mg/kg	s_r	s_R	RSD_r, %	RSD_R, %	r	R	HorRat	Outliers
4321/2025	Metal Fe oxysulfate	69.16	5.08	18.78	7.35	27.16	14.24	52.59	3.21	0/0
4031/5938	Magruder 2002-09B	116.69	21.33	29.55	18.28	25.32	59.72	82.73	3.24	0/0
6341/1436	31% $ZnSO_4$	0.32	0.67	1.51	213.01	478.56	1.88	4.23	25.15	0/0
5488/5890	Zinc oxysulfate	3.89	0.39	1.64	10.04	42.19	1.09	4.59	3.24	0/0
2818/7669	Granulated mine waste	2.73	0.60	1.19	21.89	43.64	1.67	3.34	3.17	0/1
1615/2056	Metal oxysulfate	4.39	0.98	2.80	22.27	63.90	2.73	7.85	4.99	0/0
9978/4085	Florida DAP	11.55	0.65	1.17	5.67	10.13	1.83	3.28	0.92	0/0
3313/6267	Western MAP	18.47	0.31	1.85	1.68	9.99	0.87	5.17	0.97	1/0
7161/6990	Western DAP	9.36	0.12	1.04	1.32	11.11	0.35	2.91	0.97	1/0
7999/3375	DAP from North African rock	4.00	0.50	0.71	12.49	17.68	1.40	1.98	1.36	0/0
6501/4812	NC MAP	11.74	0.56	1.34	4.75	11.44	1.56	3.76	1.04	1/0
7738/7418	China DAP	0.65	0.13	0.21	19.29	32.37	0.32	0.35	1.90	1/2
3917/8165	Magruder 2003-11	13.21	1.05	1.45	7.94	10.99	2.94	4.06	1.01	0/0
4459/8931	Magruder 2004-07	42.88	2.24	2.24	5.23	5.23	6.27	6.27	0.58	0/1
6895/4337	N-P-K blend + iron	6.75	0.31	0.80	4.61	11.86	0.87	2.24	0.99	0/0
7946/3692	Magruder 2001-03	5.42	0.11	0.55	2.11	15.68	0.32	2.38	1.26	2/0
8873/9469	N-P-K lawn product blend	0. 74	0.08	0.65	10.65	88.14	0.22	1.83	5.27	1/0
9886/9774	Organic biosolid	11.53	1.73	2.30	15.03	19.96	4.85	6.44	1.80	0/0
4626/8088	Organic mixed fertilizer + biosolid	7.83	0.57	1.71	7.22	21.84	1.58	4.79	1.86	0/0
6411/3401	Composted manure	1.03	0.54	1.00	52.29	97.20	1.50	2.80	6.10	2/0
3716/4606	Fe humate	12.44	1.80	4.40	14.46	35.39	5.04	12.33	3.23	0/0
4397/5149	Bone meal	4.28	0.30	1.10	7.08	25.77	0.85	3.09	2.00	1/0
4760/2264	Blood/feather/bone	0.59	0.47	0.88	80.12	148.92	1.32	2.45	8.59	0/0
6883/6583	Feather/bone/sulfate/gypsum	0.82	0.86	1.04	104.40	126.84	2.40	2.92	7.70	0/0
4924/7924	NIST SRM 694 Western Phos Rock	18.40	0.87	2.51	4.73	13.63	2.44	7.02	1.32	0/0
4131/1340	AFPC rock check 22	6.59	1.47	1.96	22.33	29.81	4.12	5.50	2.48	0/0
5682/8537	Peru rock	23.62	0.60	1.57	2.55	6.63	1.69	4.39	0.67	0/0
7883/3887	African rock	2.12	0.19	0.46	8.72	21.45	0.52	1.28	1.50	1/0

Table 2006.03F. Blind duplicate statistics for nickel

Sample ID	Sample description	$\bar{X}$, mg/kg	s_r	s_R	RSD_r, %	RSD_R, %	r	R	HorRat	Outliers
4321/2025	Metal Fe oxysulfate	331.92	13.07	49.17	3.94	14.81	36.59	137.68	2.22	0/0
4031/5938	Magruder 2002-09B	295.83	53.30	54.20	18.02	18.32	149.24	151.75	2.70	0/0
6341/1436	31% $ZnSO_4$	2.03	0.59	1.12	29.05	55.30	1.65	3.15	3.85	0/2
5488/5890	Zinc oxysulfate	26.60	0.94	3.99	3.55	14.99	2.64	11.16	1.54	0/0
2818/7669	Granulated mine waste	13.01	0.45	2.10	3.45	16.15	1.26	5.88	1.49	0/0
1615/2056	Metal oxysulfate	36.39	2.09	5.54	5.75	15.22	5.86	15.50	1.63	0/0
9978/4085	Florida DAP	11.14	0.73	1.62	6.55	14.58	2.04	4.55	1.31	0/0
3313/6267	Western MAP	279.34	2.61	19.63	0.93	7.03	7.31	54.97	1.03	1/1
7161/6990	Western DAP	146.57	1.56	13.52	1.06	9.22	4.37	37.85	1.22	0/0
7999/3375	DAP from North African rock	42.45	1.30	3.56	3.05	8.39	3.63	9.98	0.92	0/0
6501/4812	NC MAP	52.76	1.97	6.45	3.73	12.23	5.51	18.07	1.39	0/0
7738/7418	China DAP	6.67	0.36	1.64	5.37	24.57	1.00	4.59	2.04	1/2
3917/8165	Magruder 2003-11	101.89	7.16	15.95	7.03	15.65	20.04	44.65	1.96	0/0
4459/8931	Magruder 2004-07	1683.27	77.91	98.89	4.63	5.88	218.16	276.90	1.12	0/0
6895/4337	N-P-K blend + iron	86.88	4.02	10.12	4.63	11.65	11.26	28.33	1.43	0/0
7946/3692	Magruder 2001-03	75.25	2.45	5.76	3.26	7.65	6.87	16.12	0.92	0/0
8873/9469	N-P-K lawn product blend	6.00	0.28	0.75	4.60	12.58	0.77	2.11	1.03	2/0
9886/9774	Organic biosolid	86.22	18.37	22.31	21.30	25.88	51.42	62.47	3.16	0/0
4626/8088	Organic mixed fertilizer + biosolid	18.55	2.34	4.95	12.64	26.69	6.56	13.87	2.59	0/0
6411/3401	Composted manure	10.17	1.78	3.28	17.48	32.24	4.98	9.18	2.86	0/0
3716/4606	Fe humate	33.39	4.48	8.64	13.42	25.88	12.55	24.20	2.74	0/0
4397/5149	Bone meal	2.06	0.82	0.98	40.09	47.47	2.31	2.73	3.31	1/0
4760/2264	Blood/feather/bone	7.73	0.58	1.21	7.48	15.69	1.62	3.40	1.33	1/2
6883/6583	Feather/bone/sulfate/gypsum	2.27	0.17	1.03	7.53	45.54	0.48	2.89	3.22	2/0
4924/7924	NIST SRM 694 Western Phos Rock	129.53	2.67	14.51	2.06	11.20	7.48	40.63	1.46	1/0
4131/1340	AFPC rock check 22	10.08	1.53	2.55	15.15	25.26	4.27	7.13	2.24	0/0
5682/8537	Peru rock	14.81	0.52	1.69	3.50	11.43	1.45	4.74	1.07	2/0
7883/3887	African rock	10.18	1.28	3.20	12.54	31.47	3.57	8.97	2.79	0/0

Table 2006.03G. Blind duplicate statistics for lead

Sample ID	Sample description	$\bar{X}$, mg/kg	s_r	s_R	RSD_r, %	RSD_R, %	r	R	HorRat	Outliers
4321/2025	Metal Fe oxysulfate	119.60	76.70	76.70	64.13	64.13	214.75	214.75	8.24	0/0
4031/5938	Magruder 2002-09B	3070.11	934.61	934.61	30.44	30.44	2616.91	2616.91	6.37	0/0
6341/1436	31% $ZnSO_4$	1.49	0.35	1.18	23.50	79.21	0.98	3.31	5.25	3/0
5488/5890	Zinc oxysulfate	996.25	16.37	54.41	1.64	5.46	45.84	152.36	0.97	0/0
2818/7669	Granulated mine waste	3292.06	149.93	304.57	4.55	9.25	419.80	852.80	1.96	1/0
1615/2056	Metal oxysulfate	4075.75	666.82	666.82	16.36	16.36	1867.09	1867.09	3.57	0/0
9978/4085	Florida DAP	1.27	0.44	1.39	34.22	109.03	1.22	3.88	7.07	1/0
3313/6267	Western MAP	3.08	0.14	3.11	4.54	101.11	0.39	8.71	7.49	1/0
7161/6990	Western DAP	3.58	0.27	2.62	7.48	73.16	0.75	7.34	5.54	1/0
7999/3375	DAP from North African rock	3.81	0.60	2.62	15.89	68.85	1.69	7.34	5.26	0/0
6501/4812	NC MAP	1.14	0.20	1.49	17.22	130.84	0.55	4.16	8.34	2/0
7738/7418	China DAP	0.60	0.12	0.89	20.54	147.48	0.35	2.49	8.54	1/0
3917/8165	Magruder 2003-11	245.35	9.56	19.15	3.90	7.80	26.77	53.61	1.12	0/0
4459/8931	Magruder 2004-07	509.54	17.69	41.75	3.47	8.19	49.52	116.90	1.31	0/0
6895/4337	N-P-K blend + iron	6.52	0.38	4.37	5.89	66.99	1.07	12.23	5.55	1/0
7946/3692	Magruder 2001-03	3.42	0.54	2.55	15.77	74.78	1.51	7.15	5.62	0/0
8873/9469	N-P-K lawn product blend	0.51	0.19	0.51	37.95	99.65	0.54	1.43	5.68	1/1
9886/9774	Organic biosolid	66.29	11.41	17.75	17.21	26.78	31.95	49.71	3.15	0/0
4626/8088	Organic mixed fertilizer + biosolid	58.53	3.83	5.70	6.55	9.73	10.738	15.95	1.12	0/4
6411/3401	Composted manure	3.34	2.06	2.54	61.88	76.10	5.78	7.11	5.70	0/0
3716/4606	Fe humate	343.08	34.76	35.91	10.13	10.47	97.34	100.56	1.58	0/0
4397/5149	Bone meal	1.94	0.93	1.62	47.76	83.42	2.59	4.53	5.76	0/0
4760/2264	Blood/feather/bone	3.73	1.33	2.07	35.71	55.48	3.73	5.50	4.23	1/0
6883/6583	Feather/bone/sulfate/gypsum	3.74	1.63	3.05	43.56	81.63	4.56	8.54	6.22	0/0
4924/7924	NIST SRM 694 Western Phos Rock	10.957	0.45	5.84	4.07	53.35	1.25	16.36	4.78	1/0
4131/1340	AFPC rock check 22	15.05	0.45	6.54	2.98	43.47	1.25	18.31	4.09	1/0
5682/8537	Peru rock	4.88	0.59	3.09	12.08	63.39	1.65	8.66	5.03	0/0
7883/3887	African rock	9.24	0.86	3.46	9.36	37.41	2.42	9.68	3.27	0/0

Table 2006.03H. Blind duplicate statistics for selenium

Sample ID	Sample description	$\bar{X}$, mg/kg	s_r	s_R	RSD_r, %	RSD_R, %	r	R	HorRat	Outliers
4321/2025	Metal Fe oxysulfate	6.96	0.87	7.42	12.43	106.53	2.42	20.77	8.92	1/0
4031/5938	Magruder 2002-09B	31.03	4.39	24.27	14.15	78.23	12.29	67.96	8.20	0/0
6341/1436	31% $ZnSO_4$	4.71	0.66	3.19	13.97	67.62	1.84	8.92	5.34	0/0
5488/5890	Zinc oxysulfate	4.62	0.47	2.61	10.08	56.44	1.30	7.30	4.44	1/0
2818/7669	Granulated mine waste	28.40	1.35	21.12	4.77	74.36	3.79	59.13	7.69	1/0
1615/2056	Metal oxysulfate	25.90	2.86	14.40	11.05	55.61	8.02	40.32	5.67	0/0
9978/4085	Florida DAP	1.50	0.36	1.35	24.25	90.06	1.02	3.78	5.98	1/0
3313/6267	Western MAP	1.40	1.17	1.63	83.34	116.84	3.26	4.57	7.68	0/0
7161/6990	Western DAP	1.13	0.22	1.33	19.22	118.16	0.61	3.73	7.52	0/0
7999/3375	DAP from North African rock	1.73	0.82	1.80	47.46	103.86	2.30	5.04	7.05	0/0
6501/4812	NC MAP	0.98	0.63	1.03	63.86	105.30	1.75	2.89	6.56	0/1
7738/7418	China DAP	1.02	0.41	0.97	39.68	94.95	1.13	2.71	5.95	0/2
3917/8165	Magruder 2003-11	3.20	1.35	3.00	42.10	93.68	3.78	8.40	6.98	0/0
4459/8931	Magruder 2004-07	257.17	7.44	18.50	2.89	7.19	20.82	51.80	1.04	1/0
6895/4337	N-P-K blend + iron	1.88	1.10	2.13	58.35	113.49	3.07	5.96	7.80	0/0
7946/3692	Magruder 2001-03	0.67	0.82	1.67	121.76	249.30	2.29	4.68	14.68	0/0
8873/9469	N-P-K lawn product blend	0.25	0.19	1.58	75.67	634.02	0.53	4.43	32.16	1/0
9886/9774	Organic biosolid	9.47	0.81	2.42	8.57	25.55	2.27	6.77	2.24	1/0
4626/8088	Organic mixed fertilizer + biosolid	2.05	1.09	3.80	53.30	185.24	3.06	10.64	12.90	0/0
6411/3401	Composted manure	1.69	1.38	1.48	81.29	87.14	3.86	4.13	5.90	0/1
3716/4606	Fe humate	4.73	0.73	5.42	15.45	114.63	2.05	15.18	9.05	0/0
4397/5149	Bone meal	1.71	0.39	2.87	22.91	167.51	1.10	8.02	11.35	2/0
4760/2264	Blood/feather/bone	3.34	1.52	2.60	45.36	77.78	4.25	7.28	5.83	0/0
6883/6583	Feather/bone/sulfate/gypsum	2.19	0.65	2.62	29.51	119.62	1.81	7.33	8.41	1/0
4924/7924	NIST SRM 694 Western Phos Rock	13.56	1.24	1.85	9.14	13.65	3.47	5.18	1.26	0/0
4131/1340	AFPC rock check 22	1.59	0.69	1.00	43.72	62.79	1.94	2.79	4.21	0/0
5682/8537	Peru rock	5.18	0.49	1.67	9.49	32.19	1.38	4.67	2.58	1/0
7883/3887	African rock	2.84	1.11	2.40	39.14	84.35	3.11	6.71	6.17	0/0

volumetric flask, dilute to volume with H_2O, mix, and transfer to an acid-washed polypropylene bottle.

(**h**) *Sampler wash solution, 1% HNO_3.*—Dilute 10 mL of trace metal grade HNO_3 to 1000 mL with H_2O.

H. Determination

Analyze test solutions using an ICP-OES instrument calibrated with standard solutions. Insert a 10 mg/kg working standard or other suitable quality control solution every 10 test portions, to monitor for instrument drift. The inclusion of a digestion blank, a sample duplicate, and known reference materials is highly encouraged.

I. Calculations

$$\text{Element, mg / kg} = \frac{\text{(instrument concentration, mg / L)(100 mL)(1 L / 1000 mL)}}{\text{(sample weight, g)(1 kg / 1000 g)}}$$

where 100 mL assumes the microwave digest is diluted to 100 mL.

Reference: J. AOAC Int. 89, 1447(2006).

2.4.20

AOAC Official Method 959.03
Urea in Fertilizers

Urease Method
First Action 1959
Final Action 1960

A. Reagent

Neutral urease solution.—Use fresh commercial 1% urease solution, or dissolve 1 g urease powder in 100 mL H_2O, or shake 1 g jack bean meal with 100 mL H_2O 5 min. Transfer 10 mL solution to 250 mL Erlenmeyer, dilute with 50 mL H_2O, and add 4 drops methyl purple (available from Fisher Scientific Co.; No. SI9-500). Titrate with 0.1M HCl to reddish purple; then back-titrate to green with 0.1M NaOH. From difference in mL, calculate volume 0.1M HCl required to neutralize remainder of solution (usually ca 2.5 mL/100 mL), add this amount of acid, and shake well.

Verify enzyme activity of urease source periodically. Discard any source which does not produce solution capable of hydrolyzing 0.1 g urea/20 mL solution.

B. Determination

Weigh 10 ± 0.01 g test portion (≤1.0 g of urea) and transfer to 15 cm Whatman No. 12 fluted filter paper. Leach with ca 300 mL H_2O into 500 mL volumetric flask. Add 75–100 mL saturated $Ba(OH)_2$ solution to precipitate phosphates. Let settle and test for complete precipitation with few drops saturated $Ba(OH)_2$ solution. Add 20 mL 10% Na_2CO_3 solution to precipitate excess Ba and any soluble Ca salts. Let settle and test for complete precipitation. Dilute to volume, mix, and filter through 15 cm Whatman No. 12 fluted paper. Transfer 50 mL aliquot (equivalent to 1 g test portion) to 200 or 250 mL Erlenmeyer and add 1–2 drops of methyl purple. Acidify with 2M HCl and add 2–3 drops excess. Neutralize solution with 0.1M NaOH to first change in color of indicator. Add 20 mL neutral urease solution, close flask with rubber stopper, and let stand 1 h at 20°–25°C. Cool flask in ice-water slurry and titrate at once with 0.1M HCl to full purple; then add ca 5 mL excess. Record total volume added. Back-titrate excess HCl with 0.1M NaOH to neutral end point.

$$\text{Percent urea} = \frac{(\text{mL 0.1M HCl} - \text{mL 0.1M NaOH}) \times 0.3003}{\text{g test portion}}$$

References: *Ind. Eng. Chem. Anal. Ed.* **7,** 259(1935).
JAOAC **41,** 637(1958); **42,** 494(1959);
43, 123(1960).

CAS-57-13-6 (urea)

OPEN FOR COMMENTS

Proposed Modification to AOAC Official Method 959.03 Urea In Fertilizers

July 25, 2016: The AOAC Research Institute announces a notification of a proposed change in status of an AOAC Final Action Official Method 959.03 [Final Action 1960]. The open public comment period for the proposed modification of AOAC Official Method 959.03 will be posted for a minimum of 30 days. The comment period opens on **Monday, July 25, 2016** and closes **Wednesday, August 17, 2016.** Comments will be compiled, reviewed, and intended to obtain input on the proposed modification. The documents may be revised if necessary, based on comments received. Any interested party may submit comments.

A modification to AOAC Official Method 959.03: Urea in Fertilizers [Final Action 1960] is being proposed. Please see the following information regarding the modification:

Summary of Proposed Modification or Extension

By providing the statistical data, it will prove that the AOAC Official Method 959.03 is not suitable for urea-formaldehyde condensate fertilizer products and an editorial/clerical change is proposed.

Summary of how the proposed modification will be evaluated

By providing statistical data measuring the free urea contents in at least 8 different samples of these fertilizers by comparing the results of their free urea contents using both AOAC Official Method 959.03 (Urease Method) and AOAC Official Method 2003.14 Urea in Water-Soluble Urea-Formaldehyde Fertilizer Products and in Aqueous Urea Solutions [Final Action 2009].

Comment Process

AOAC requests that the following guidelines be observed in providing comments regarding the above modification:

1. Please specify if the comment is an editorial, content, or disagreement comment.
2. Provide rationale as to why the comments should be considered.
3. All comments are due within 30 days of the initial posting date (**deadline**: **Wednesday, August 17, 2016**). AOAC reserves the right to not to accept comments received after the deadline.
4. Editorial comments provide additional clarification or correct typographical errors. Please suggest alternative wording or typographical corrections.
5. Content-related comments provide technical clarity and comprehensiveness. Please suggest the appropriate technical language. Documents will be reviewed by AOAC for technical accuracy and clarity.
6. Disagreement comments reflect a perspective on content documented/undocumented in the drafts. Please provide language that document the perspective or position.

To provide comments and review the proposed modification of AOAC Official Method 959.03, please use the link at HTTPS://FORM.JOTFORM.COM/61666298869175.

Evaluation of the Determination of Free Urea in Water-Soluble Liquid Fertilizers containing Urea and Ureaforms by Urease Method and by HPLC Methods

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Abstract

Currently there are three AOAC Official Methods for the determination of urea in fertilizers.

AOAC Official Method 959.03, Urea in Fertilizers, Urease Method, First Action 1959, Final Action 1960. This method is based on the use of fresh commercial 1% urease solution, or preparation of such solution from urease powder in water, or from jack bean meal in water[1].

AOAC official Method 983.01, Urea and Methyleneureas (Water-Soluble) in Fertilizers, First Action 1983, Final Action 1984, is based on liquid chromatography with refractive index detector using water as mobile phase and an ODS column[1].

AOAC Official Method 2003-14, Determination of Urea in Water-Soluble Urea-Formaldehyde Fertilizer Products and in Aqueous Urea Solutions, First Action 2003, Final Action 2008, is also based on liquid chromatography with UV detector using 85%:15% Acetonitrile: Water as mobile phase and a propylamine column[2].

The Urea Method, AOAC Official 959.03 is very much dependent to the nature of the urease enzyme. The method was developed in 1960 and used for simple urea fertilizer solutions. With the advent of complex fertilizers compositions, especially with the class of liquid Triazone Fertilizers, and water-soluble ureaforms, the analyses of free urea in these fertilizers by the urease method is often inaccurate and inconsistent.

The AOAC Official Method 983.01 is not always reliable due to the interference of some of the components of these fertilizers, and due to the fact that the use of water as mobile phase does not always separate the free urea from other components[3].

The AOAC Official Method 2003.14 was subjected to Ring Test Studies and showed it could be used for the determination of "free urea" in these classes of fertilizers with good accuracy and precision[3].

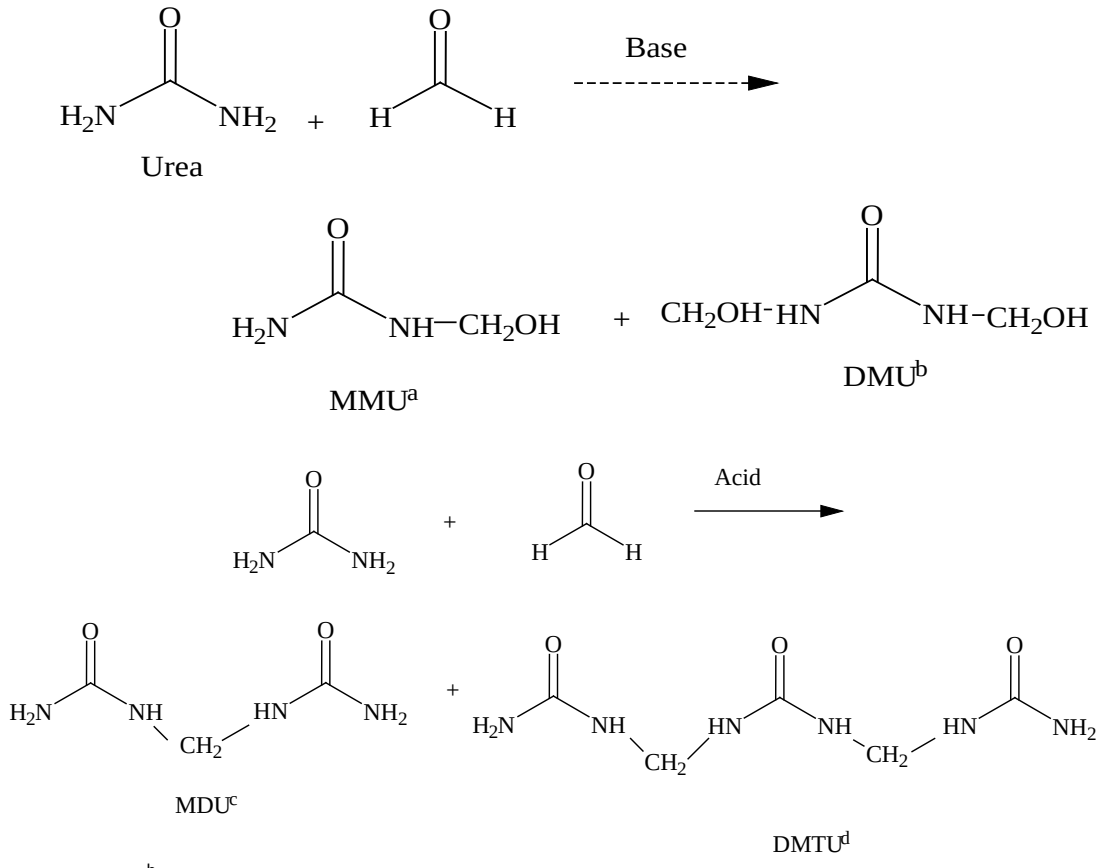
Introduction

The critical comparison of some currently manufactured commercial slow and controlled release nitrogen (SRN, and CRN)-containing fertilizers, especially liquid products, whether by means of technical

data sheets, product labels or otherwise, is unfortunately not always as an easy task for the potential user. In this matter, particular attention must be paid to the methods used by various manufacturers for the determination and reporting of the amount of SRN, and CRN present.

The SRN in most commercial liquid SRN-containing fertilizers presently manufacture in the United States, Canada, and Europe is comprised of the water-soluble reaction products of urea with formaldehyde, or from the reaction products of urea, formaldehyde and ammonia.

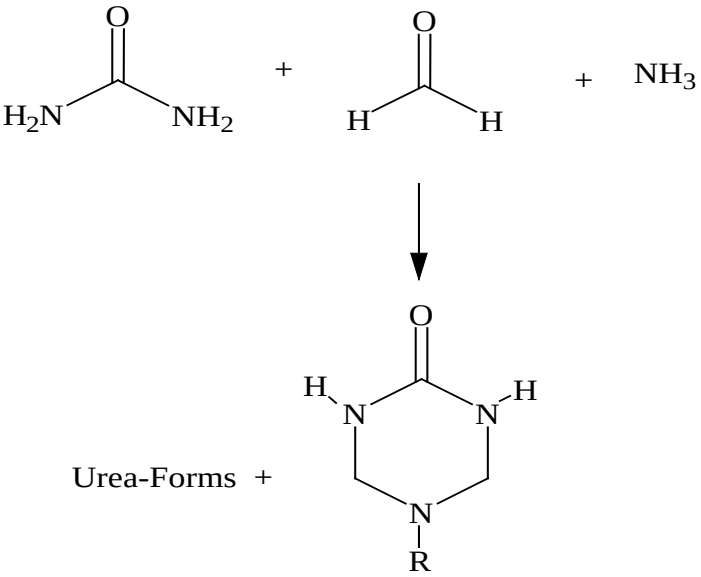
In the reaction of urea and formaldehyde, based on the nature of the catalysts, whether an acid catalyzed reaction, or a based catalyzed reaction, different urea-formaldehyde adducts (ureaforms) will form and the resulting product will be either liquid or solid[4].



[a] monomethylourea; [b]dimethylolurea;[c]methylenediurea; [d]dimethylenetriurea

Reaction of urea and formaldehyde is not 100% and some unreacted urea will remain in the final product. If the reaction product is a solid, the amount of unreacted and free urea could be determined by AOAC Official Method 945.01 (Water-Soluble Nitrogen), and AOAC Official Method 955.05 (Nitrogen Activity Index). Determination of water-insoluble nitrogen in mixed fertilizers has been studied by Katz, et.al.[5,6]

In the reaction of urea, formaldehyde, and ammonia, a highly water soluble ring structure known as urea-triazone predominantly forms.

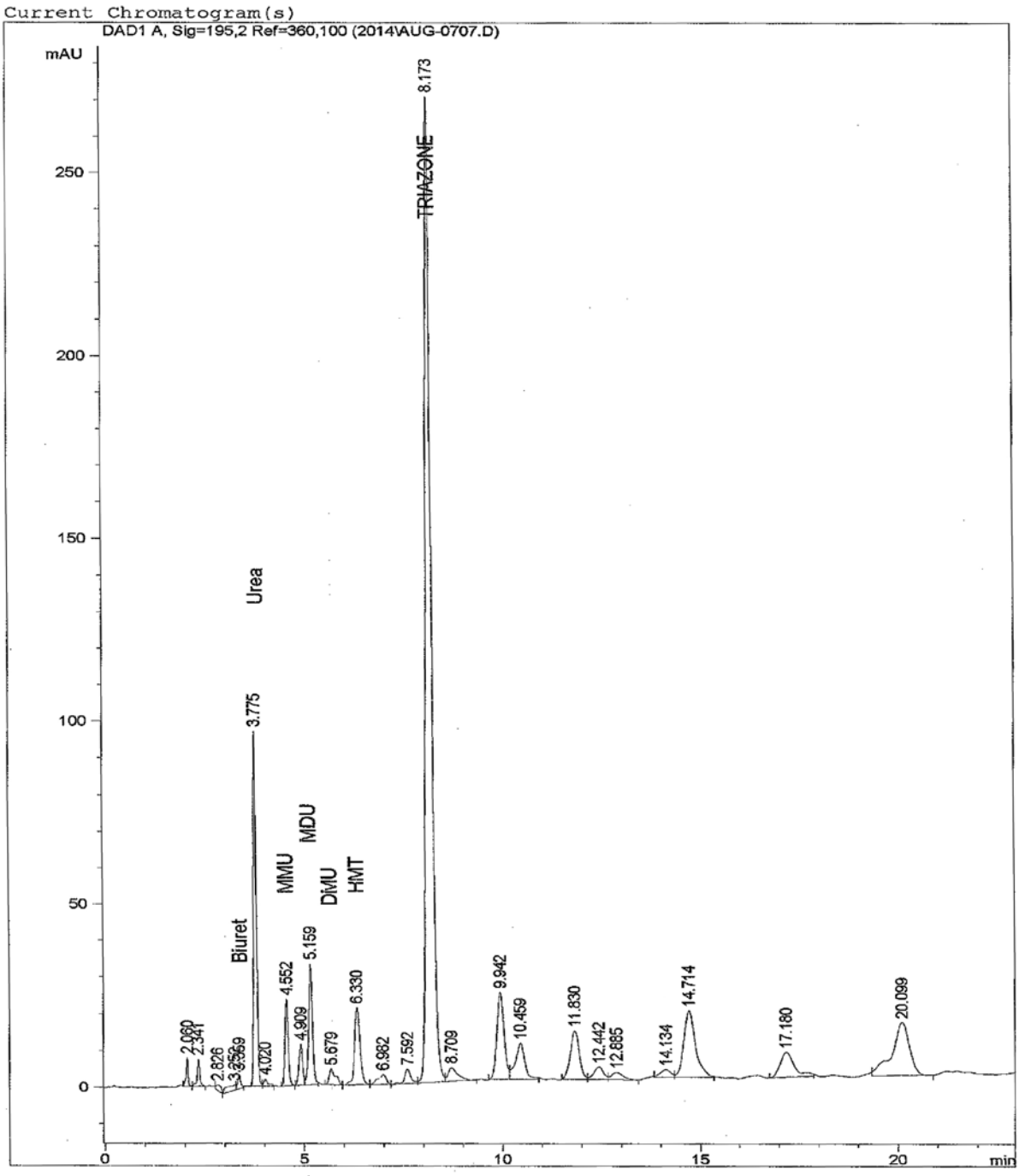


$R = H, CH_3, CH_2OH, CH_2NHCONH_2$

In most of these products produced from the reaction of urea and formaldehyde, or urea-formaldehyde-ammonia, the reaction products contain SRN in the form of aqueous solutions of water-soluble organic nitrogen species and some unreacted, free urea. The unreacted or free urea provides readily available nitrogen and therefore should be deducted from the slowly released nitrogen portion.

Quantitative analysis of these SRN fertilizer solutions for all of the reacted nitrogen forms in these fertilizers is time consuming and impractical or even impossible. The complex composition of an example of a fertilizer produced from the reaction of urea, formaldehyde, and ammonia is shown in Figure - 1 below:

Figure 1
Liquid Chromatography of Triazone Fertilizer



(MMU = monomethylolurea; MDU = methylenediurea; DMU = dimethylolurea; and HMT = hexamethylenetetramine)

Therefore, the direct determination of the amount of SRN by summingthe amounts of reacted organic N from various forms is not a viable option. Consequently, SRN content is most often determined as the difference of the unreacted free urea subtracted from total the N, both of which can be fairly easily determined. The remainder from this subtraction is the combined reacted organic N (or SRN), except in cases where some other nitrogen form such as nitrate may have been added after the reaction in order to make a mixed fertilizer.

Currently, there are three AOAC Official Methods available for the determination of the free and unreacted urea in liquid and water-soluble urea containing fertilizers.

The AOAC Official Method 959.03 is the most well-known and the oldest method for urea determination in fertilizers. This method, which was introduced in 1959, is based on the quantitative hydrolysis of urea to ammonia by means of urease enzyme. The amount of ammonia generated by hydrolysis is subsequently determined and reported as equivalent urea or urea N by a simple titration. With most simple and conventional (i.e. non-SRN-containing) liquid fertilizers, the urease method works very well. Urea-based methods are well accepted and usually quite suitable for urea determinations in most urea-containing fertilizers, however, they have been found to demonstrate significant problems, particularly with incomplete recovery of urea, when applied to such determinations in Urea-Formaldehyde (UF) fertilizer solutions. Comparisons of the HPLC methods, particularly the AOAC Official Method 2003.14 with results from the urease method, frequently produced results that were not in agreement with each other. Further study also showed that urea results from the urease methods often were not reproducible. Thus a controlled study, which is the principle subject of this article, was carried out to confirm and elaborate upon these observations, and to suggest some likely reasons for the reduced effectiveness of the urease method with UF fertilizers.

Experimental

Materials

Twelve of the most commonly available commercial liquid urea-based fertilizers were selected for the determination of their free urea contents using the three aforementioned methods. These commercial samples were:

1. LF3060 (30-0-0), Koch Agronomic Services, LLC, 4111 E. 37th St. N, Wichita, KS 67220, USA
2. CoRoN (28-0-0), Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017
3. Arclin 28-0-0, Arclin Resins, Research & Technology, 4754 28th St., Springfield, OR 97477
4. Greenfeed 27-0-0 (light Green liquid), Plant Food Company, 38 Hightstown-Cranbury Station Road, Cranbury, NJ 08512
5. Greenfeed 27-0-0 (Dark Green liquid), Plant Food Company, 38 Hightstown-Cranbury Station Road, Cranbury, NJ 08512
6. NDemand 30-0-0, Wilbur-Ellis Agribusiness, 3300 S. Parker Road, Suite 500, Aurora, CO 80014, USA
7. NDemand 30L, Wilbur-Ellis Agribusiness, 3300 S. Parker Road, Suite 500, Aurora, CO 80014, USA
8. Gradual N (30-0-0), Winfield Solutions, LLC, 1080 County Road West, Shoreview, MN 55126
9. N-28 clear Fertilizer (28-0-0), Advachem, Route de Wallonie, Darse d'Hautrage, BE 7334 Hautrage
10. N-Sure (28-0-0), Tessenderlo Kerley, Inc., 2255 N. 44th St., Suite 300, Phoenix, AZ
11. Trisert NB (26-0-0), Tessenderlo Kerley, Inc., 2255 N. 44th St., Suite 300, Phoenix, AZ
12. Formolene Plus (30-0-0), Tessenderlo Kerley, Inc., 2255 N. 44th St., Suite 300, Phoenix, AZ

These fertilizers are liquid and were used without any further modifications, unless specified in the method, (i.e., dilution).

The abovetwelve commercially available U-F liquid fertilizers were tested by the authors' lab (Lab 1) and by an independent lab (Lab 2) using AOAC Official Method 2003.14 for the determination of their unreacted (free) urea by HPLC. Each sample was analyzed for its free urea content in duplicate. The inter laboratory results were compared to each other for the consistency of this method for the determination of free urea contents in these fertilizers by comparing each value obtained from the means of duplicate analyses. Analytical results, means and standard deviations are shown in Table 2.

The same materials were also analyzed by a commercial lab using the AOAC Official Method 953.01 (Urease Method) for the determination of their free urea contents. Each sample was analyzed in duplicate and their means were used for comparisons with the means of those results obtained by Lab 1, and Lab 2 using the AOAC Official method 2003.14. The commercial lab used powdered form of urease made from Jack bean meal.

In addition, different sources and forms of urease enzyme were tested to understand what effects (if any) of the urease enzyme has on the determination of free urea contents of this class of fertilizers.

Analytical methods

AOAC Official Method 2003.14[2]

A. *Principle*

A precisely weighed portion of homogenous urea or homogenous water-soluble urea-containing liquid UF fertilizer sample was diluted to volume with solvent of the same composition used as the liquid chromatographic mobile phase. Ureawas determined via high performance liquid chromatography (HPLC) employing ultraviolet wavelength detection. The area beneath the absorption peak due to urea in the sample was compared with the area beneath the absorption peak for urea in an external standard solution prepared with pure urea in the mobile phase solvent.

B. *Apparatus*

(a) *Liquid Chromatograph.* – Requires a high performance liquid chromatograph capable of isocratic delivery of mobile phase at 2 ml/min at 204 bars (3000 psig) and having a UV absorption detector capable of stable operation at 195 nm (Acetonitrile and water absorption cutoff). Instrument operating conditions are listed in Table 1:

Table - 1
HPLC Operating Conditions and Settings

Operating conditions	Setting
Flow Rate	1.3ml/min
Mobile Phase Temperature	Ambient
Column temperature	35 ^{0}C
Detector Wavelength	195nm
Injection Volume	10 µl

For best precision a fixed volume sample loop is preferred to syringe injection of samples and standards. To analyze fertilizer solution for urea, allocate 14 minutes for each injection, 12 minutes for run time and 2 minutes for post run time. For more complex fertilizer solutions, allocate a total of 43 minutes for each injection, which includes 23 min of run time and 20 min of post-run timeto avoid overlapping.

(b) *HPLC Column* – The chromatography column is a 4.6 mm ID x 250 mm length amino propyl (NH_2) column, 5µ particle size. Examples are Phenomenex amino propyl column Spherex, Part # 00G-00051-E0, or Thermo Scientific Hypersil APS-2 column, Part # 30703-254630. Before use, new columns must be conditioned as described in Section (c) hereof.

(c) If the HPLC column is new or has not been in service for about a week or more, it must be conditioned as follows before using. A conditioned column will usually last for about a year depending on the number of analyses. This conditioning step is only applied to new columns or columns that are not used on a weekly basis:

(1) Using the HPLC instrument, wash the column for about two to four hours at room temperature with HPLC-grade isopropanol at a flow rate which will maintain at least 200 bars column back pressure (typically about 1 ml/min).
(2) Follow step 1 with a second column wash with 100% HPLC-grade acetonitrile at room temperature for 4 hours.
(3) Follow step 2 with a final column wash using mobile phase solution [see C(a)] at 1.3 ml/min and normal analytical operating conditions. This wash should continue for 2 hours or as long thereafter as needed to obtain a stable base line.

C. *Reagents*

(a) *Mobile Phase* – 85% (v/v) acetonitrile with water. Use LC grade acetonitrile having 190 nm maximum UV cutoff and LC grade water.
(b) *Urea standard-ACS reagent, 99-100.5%, Sigma-Aldrich Chemical Company, Milwaukee, WI.*

D. External Standard

Urea Standard - Accurately weigh amounts of 100% pure urea of approximately 0.0150g and 0.0300g into separate 100 ml volumetric flasks. Use ultrasound for three minutes to dissolve and then dilute to volume with mobile phase solution. Shake well.

E. Sample Preparation

Accurately weigh a portion of uniform sample containing an estimated amount of free urea between 0.0150g and 0.0300g into a 100 ml volumetric flask. Use ultrasound for three minutes to dissolve and then dilute to volume with mobile phase solution and shake well to assure homogeneity. (Note: For solid samples or fluids containing substantial undissolved solids, ultrasound for 5 minutes shake periodically over a period of about 15 minutes to ensure that all urea has an opportunity to dissolve.) Filter a portion through a 0.45 µm porosity (or finer) filter before injecting onto HPLC column. Samples should be analyzed on the same day as prepared.

Determination

(a) Inject 10 µl of each urea standard until two consecutive injections of each give the same peak area within ± 2% for the same standard. Average the peak areas for the accepted standard determinations.

(b) Inject 10 µl of prepared sample. Identify the urea peak by retention time relative to a urea standard and note if the peak area falls within the range of the high and low standards. If not, prepare a new sample with the weight adjusted to permit peak to fall within the standard range.

(c) Perform sufficient sample injections (minimum of two) from the same sample flask such that at least two consecutive determinations yield peak areas which agree with each other to a precision of at least ± 2%.[2] Determine the average value of agreeing peak areas.

Calculations

Calculate the average instrument urea working standard response from n standards as:

$$\text{Urea Factor} = \left[\sum \left(\frac{AP_r}{W_r}\right)\right] / n$$

Where AP is the average response of 2 or more agreeing peak area for the working standard r, W_r is the weight of urea in the 100 ml working standard and n is the number of agreeing working standards used in the calculation.

AOAC Official Method 959.03[1]

Reagents

Either fresh commercial 1% urease solution was used or the urease solution was prepared by dissolving 1 gram of urease powder in 100 ml of distilled water, or one gram jack bean meal was transferred into 100 ml of distilled water and shaken for 5 minutes. Ten ml of this solution was transferred into a 250 ml Erlenmeyer flask, and diluted with 50 ml distilled water. The enzyme activity was determined by titration with 0.1 N HCl in the presence of methyl purple (Fisher Scientific, Suwanee, GA) to a reddish purple color, then back titrated with 0.1N NaOH to a green color. The amount of 0.1 N hydrochloric acid required to neutralize the remainder of solution was calculated and added to this solution. The enzyme

activity was verified periodically, and any source which did not produce solution capable of hydrolyzing 0.1 g urea/20 mL solution was discarded.

Determination

Ten grams of each liquid ureaformaldehyde fertilizer sample (containing ≤1.0g of urea) was transferred to a 15 cm Whatman No. 12 fluted filter paper (Fisher Scientific). This was leached with approximately 300 ml of de-ionized water (D-H_2O) into a 500 ml volumetric flask. Next, 75-100 ml of saturated barium hydroxide, $Ba(OH)_2$(Fisher Scientific) was added to precipitate out any phosphate present. Then, 20 ml of 10% sodium carbonate, Na_2CO_3(Fisher Scientific) solution was added to precipitate any excess Ba. This was diluted to volume, mixed, and filtered through a 15 cm Whatman No. 12 fluted filter paper. Then, 50 ml of the filtrate was transferred to a 250 ml Erlenmeyer flask, and 2-4 drops of methyl purple was added followed by addition of 2N HCl to form a reddish purple color (acidic). This was neutralized with 0.1 N NaOH to a green color. Finally, 20 ml of neutral urease solution was added and the flask closed with a rubber stopper. After one hour storage at room temperature, the flask was cooled in an ice-water bath and its content was titrated with 0.1N HClto a full purple color, and then a 5ml excess of 0.1 N HCl was added. Excess HCl was back titrated with 0.1 N NaOH to a neutral end point (green color).

Percent urea calculated as follow:

$$\%\ Urea = \frac{[ml\ 0.1\ N\ HCl - ml\ 0.1\ N\ NaOH)x\ 0.3003^{*}]}{g\ sample}$$

*0.3003 = this factor takes into account the molecular weight of urea, the conversion of the milliequivalent result of V x N, and the conversion to %.

Results and Discussion

The free urea contents of each fertilizer sample were measured in duplicates by the AOAC Official Method 2003.14 by Lab 1 and Lab 2.

The results of analyses and t-test calculation of the twelve commercial liquid fertilizer sampleslisted above from Lab 1 and Lab 2 by the AOAC Official Method 2003.14 are listed in Table 2.

Table – 2
Analyses of Twelve Fertilizer Samples for Urea by AOAC Method 2003.14

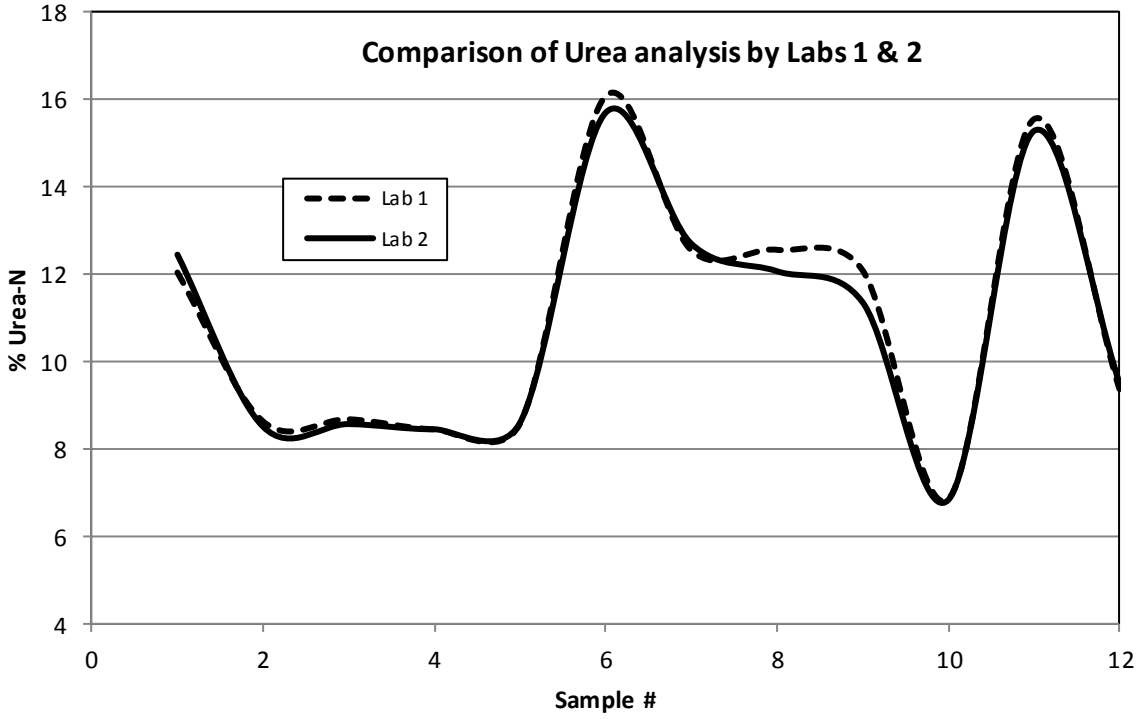
Fertilizer Sample	Lab ID	Total –N*	% Urea-N		Mean	Std. dev.
			Lab 1	Lab 2		
1	26-068-03	29.89	12.04	12.45	12.25	**0.21**
2	26-051-01	28.54	8.63	8.51	8.57	**0.06**
3	26-068-02	28.44	8.68	8.57	8.63	**0.05**
4	26-076-07	28.33	8.45	8.45	8.45	**0.00**
5	26-076-06	28.47	8.58	8.60	8.59	**0.01**
6	26-076-08	24.95	16.08	15.70	15.89	**0.19**
7	26-079-01	30.78	12.55	12.70	12.63	**0.07**
8	26-086-04	30.06	12.56	12.07	12.32	**0.25**
9	26-103-02	28.01	12.09	11.36	11.73	**0.37**
10	26-103-03	28.13	6.85	6.83	6.84	**0.01**
11	26-100-03	26.36	15.55	15.28	15.42	**0.14**
12	26-010-02	29.87	9.37	9.51	9.44	**0.07**
Average		$\bar{X}$	10.95	10.84	-	**-**
Standard Deviation		σ_x	2.97	2.87	-	**-**
Number of samples		n	12	12	-	**-**
Variance		σ^2	8.80	8.21	-	**-**
Pearson Correlation		0.99	-	-	-	**-**
T Stat		1.30	-	-	-	**-**
P(T<t) one-tail		0.11	-	-	-	**-**
t Critical one-tail		1.80	-	-	-	**-**
P(T<=t) two-tail		0.22	-	-	-	**-**
T Critical two-tail		2.20	-	-	-	**-**

* Note: Total-N includes other forms of nitrogen in addition to unreacted (free) urea

A statistical comparison (Students t-test, df, and, P) indicated that the results from two labs were statistically similar.

The results for the analyses of the Free and Unreacted urea in these twelve fertilizers by two independent Labs using AOAC Official Method 2003.14 are shown graphically as well in Figure 2.

Figure 2
Schematically comparisons of HPLC Results by two Labs



When the same samples were analyzed by urease method based on the AOAC Official Method 959.03,the results are much different and in this case showed a high biased in ten of the analyses and low biased in the remaining two analyses. The results and the differences between the results from the urease method and the HPLC method are shown in Table 3.The lowest % Urea-N difference from the two methods shown in Table 3is -0.45 and the highest % Urea-N difference is 2.42. These results were obtained by a commercial lab using powdered form of urease made from Jack Bean meal.

Table-3
Urease Analyses of Twelve Fertilizer Samples & Comparisons with the HPLC Results for Urea

		% Urea-N	%Urea-N	% Urea-N
Total-N	**Fertilizer Sample**	**Urease Method AOAC 959.03**	**HPLC Method AOAC 2003.14 (Means from two Labs)**	**Difference Urease-HPLC (Mean)**
29.89	**1**	13.26	12.25	***+1.02***
28.54	**2**	9.87	8.57	***+1.30***
28.44	**3**	10.06	8.63	***+1.44***
28.33	**4**	9.33	8.45	***+0.88***
28.47	**5**	9.91	8.59	***+1.32***
24.95	**6**	15.44	15.89	***-0.45***
30.78	**7**	14.81	12.63	***+2.19***
30.06	**8**	14.08	12.32	***+1.77***
28.01	**9**	8.89	11.37	***-2.48***
28.13	**10**	8.94	6.84	***+2.10***
26.36	**11**	16.18	15.42	***+0.77***
29.87	**12**	11.86	9.44	***+2.42***

In another series of studies supplies of seven different analytical quality urease enzyme sources were obtained from five well-known suppliers of laboratory chemicals which are identified as numbers 1 through 7. It is highly probable that one or more of these common sources are found in a large number of fertilizer analytical laboratories. Represented are four brands of urease powder, one brand of urease tablets and two brands of urease-glycerol extract solution. Only one supplier provided the information regarding the urease activity as part of its product label.

Tests using the urease enzymes in this study were carried out in accordance with the AOAC Official Method 959.03, with one exception where anaccommodation for the use of urease tablets or glycerol extract was required. The AOAC urease method specifies the use of a 1% aqueous solution of urease powder or Jack bean meal.

According to the AOAC urease method, 20ml of 1% solution of urease should be used and also the urease activity should be such that it will hydrolyze at least 0.1 gram of urea under the conditions specified in the method. The weight of fertilizer sample should also be such that the amount of urea in the final working volume does not exceed 0.1 gram.

In one series of tests, pure urea (99.4%) was used. The weights of urea samples used were 0.1g, 0.3g, 0.6 g, and 1.0 gram. These urea samples were analyzed using seven ureasematerials from different sources. The degrees of urea hydrolysis with the seven different urease materials are shown in Table 4.

Table-4
Analyses of Urea using Different Sources of Urease

Urease Source	Urease Amount	% Hydrolysis of urea from the amount used			
		0.1 g	0.3 g	0.6 g	1.0 g
1	20 ml (1% Sol.)	100.1	100.1	99.7	99.9
2	10 ml Glycerol Ext.	99.9	99.7	99.7	69.9
2	20 ml Glycerol Ext.	99.9	99.7	99.7	99.4
3	20 ml (1% Sol.)	99.6	99.7	97.6	62.7
4	20 ml (1% Sol.)	99.3	99.7	83.2	52.6
5	2 tablets	100	98.6	69.3	45.5
6	20 ml Glycerol Ext.	99.9	99.9	99.7	99.5
7	20 ml (1% Sol.)	100.3	100.1	99.9	99.6

In the amount used, all seven urease sourceshydrolyzed up to about 0.3 grams, thus exceeding the AOAC method requirements. However the urease tablets (source No. 5) begins to fade in effectiveness at 0.3 grams urea, with only 98.6% hydrolyzed, and two urease powders (No. 3and No. 4) begin to fail at about 0.6 grams of urea. The two other powder samples (No. 1 and No. 7) and the two urease glycerol extracts (No. 2 and No. 6) in 20 ml increments all hydrolyzed up to 1.0 gram of urea. Powder No.7 was known from the manufacturer's label to have a very high urease activity. From the results, it appears that sources 1, 2, and 6 were high activity urease samples. These data show that the AOAC Official Method 959.03 works well for pure urea when applied within the limits of the method. However, if the amount of urea exceeds the recommended amount in the method, there are inconsistencies with the results and some urease sources work better than others.

The inconsistencies with the Urease Method are obvious when the method applies to the more complex fertilizer samples, namely, the Urea-Triazone liquid solution containing mixed compositions of urea-forms and triazone moieties (Table 2).

More tests were done to further illustrate these inconsistencies even further.

In the following sets of experiments, five Urea-Triazone fertilizers from four different manufacturers were analyzed for their claim of %SRN. The percentage of free and unreacted urea in these samples was analyzed by the HPLC and by the urease methodusing powdered urease enzyme from Jack bean meal. The results are shown in Table 5.

Table 5
Comparisons of the Results by HPLC (2003-14) and by Urease Method(Powdered Sample)

Product % Total N	%SRN Claimed	SRN Determination	
		Method	
		HPLC (2003-14)	Urease
28-0-0	**72**	72	88
30-0-0	**60**	60	77
30-0-0	**50**	50	68
28-0-0	**75**	50	70
30-0-0	**85**	46	80

The results by the urease method for the SRN contents (i.e., Total N minus Free Urea-N) of these known fertilizers were always different from the claimed amount, while the results by HPLC were on target for three of five samples and off for two samples.

The following results further illustrate the inconsistencies of the Urease Method when applied to this class of fertilizers[7]. Comparisons of the urea results obtained with the different urease sources listed in Table 4 with the HPLC Method 2003.14 are shown in Table6. The results are for four different aqueous Urea-Triazone fertilizers and are shown in three ways, (1) as the absolute weight of urea found in each sample volume by both methods, (2) as the weight percent urea in the fertilizers, and (3) as the percent recovery of urea by the Urease Method relative to 100% recovery by the HPLC Method.

These results clearly show that the Urease Method using urease sources 1, 3 and 4 finds less urea in all four fertilizer samples in comparisons with the results from the HPLC Method. The differences are substantial. For example, sometimes less than half as much urea is recovered by the Urease Method. For the source No. 2, it makes a significant difference whether 10 ml of Glycerol extract or 20 ml of glycerol extract was used. Source No. 5 (Urease tablets) showed the poorest recovery results. All of these urease sources should normally be expected to totally hydrolyze the urea in these fertilizers. However, four of these Urease sources (Nos. 1, 3, 4, and 5) plus 10 ml of the source No.2 Glycerol extract significantly failed to hydrolyze all of the urea in any of these four fertilizer samples.

Table-6
AOAC 959.03 (Urease Method) vs. AOAC 2003.14 (HPLC) Results for Analyses of Unreacted Urea in Urea-Triazone Liquid Fertilizers

amount used	Sample	Urease	HPLC	Urease	HPLC	Urease vs HPLC
1	A	0.20	0.34	20.2	33.7	60
20ml 1% Solution	B	0.11	0.16	10.9	16.1	68
	C	0.24	0.32	23.5	32.1	73
	D	0.27	0.35	27.3	35.1	78
2	A	0.17	0.34	16.9	33.7	50
10ml Glycerol Ext.	B	0.08	0.16	7.5	16.1	47
	C	0.14	0.32	14.2	32.1	44
	D	0.25	0.35	25.4	35.1	72
2	A	0.34	0.34	34.3	33.7	102
20ml Glycerol Ext.	B	0.17	0.16	17.0	16.1	106
	C	0.32	0.32	32.4	32.1	101
	D	0.33	0.35	33.4	35.1	95
3	A	0.15	0.34	15.0	33.7	45
20ml 1% Solution	B	0.10	0.16	9.5	16.1	59
	C	0.18	0.32	17.8	32.1	55
	D	0.32	0.35	32.4	35.1	92
4	A	0.18	0.34	18.1	33.7	54
20ml 1% Solution	B	0.11	0.16	11.0	16.1	68
	C	0.18	0.32	18.4	32.1	57
	D	0.24	0.35	23.5	35.1	67
5	A	0.17	0.16	6.9	16.1	43
Two Tablets	B	0.13	0.32	13.0	32.1	40
6	B	0.17	0.16	16.9	16.1	105
10 ml Glycerol Ext.	C	0.33	0.32	33.1	32.1	103
6	B	0.17	0.16	17.1	16.1	106
20 ml Glycerol Ext.	C	0.33	0.32	33.2	32.1	103
7	A	0.34	0.34	34.0	33.7	101
20ml 1% Solution	B	0.16	0.16	16.2	16.1	101
	C	0.32	0.32	32.3	32.1	101
	D	0.33	0.35	33.0	35.1	94

In quantitative terms, hydrolyses of urea in these four fertilizer samples by these urease sources tended to be in the range of 40-80%. Each of the urease sources should have hydrolyzed all the urea in these type of fertilizers based on the tests done using the pure urea (Table 4), within the scope of the Urease Method. Fertilizer “B” contains only about half of the urea of the other three fertilizer samples (A, C, and

D). However, even with less free, unreacted urea in the sample, recovery by these urease sources was not improved. From the data in Table 5, the lower activity urease sources failed substantially to hydrolyze all the urea in these UF fertilizers, while the high activity sources did hydrolyze all the urea. The fact that high activity sources (No. 1, 7, and 20 ml extracts of No.2) show the same percent urea hydrolyses as the amount found by HPLC analyses, support that such percentages urea are indeed present in these UF fertilizers samples. The combined performances of HPLC and the high activity urease sources indicate that the AOAC Method 959.03 is very much dependent on the type of urease.

The above data show that analyses of Urea-Formaldehyde fertilizer solutions using the urease method are not consistent. In most cases, the amount of free and unreacted urea analyzed by the urease method showed a low biased (Table 6). However, in some occasions the results showed a high bias (Table 3).

These data support the discussion that AOAC official Method 959.03 (Urease Method) is not suitable for the determination of free-unreacted urea in the Urea-Formaldehyde fertilizer solutions. The HPLC Method consistently provides more accurate analyses of unreacted urea in this class of fertilizers.

As mentioned above, there are two AOAC Official Methods by HPLC for the determination of unreacted urea in these fertilizers, namely AOAC Official Method 983.01, and AOAC Official Method 2003.14.

The advantage with AOAC 983.01 is the use of water as the mobile phase, however due to the use of water, the peak separations in the complex compositions of these fertilizers (Figure 1) is not efficient and some of the peaks might overlap with that of urea resulting in erroneous data. A collaborative study[3] by the International Standards organization (ISO-CD 18643) involving 13 laboratories for the biuret content of several fertilizers, including the water-soluble Urea Triazone fertilizers,has showed that AOAC 983.01 HPLC method results in erroneous analyses of some of these UF fertilizer solutions. The same study showed that the AOAC 2003.14 method accurately predicts the amounts ofunreacted urea in these fertilizers.

Table 8 shows the inconsistency of the AOAC Official Method 983.01 (HPLC) for the determination of biuret in the Urea-Triazone fertilizers (Grade 26-0-0). The biuret co-elutes with the free and unreacted urea in these fertilizers and their peaks overlap resulting in erroneous analytical data.

Table 7

Comparisons of the analytical results for Grade 26-0-0 by AOAC 983.1 & AOA 2003.14

Sample ID	*Samples Analyzed by Lab-1*	Grade	% Biuret Test 1	% Biuret Test 2	% Biuret Test 3	% Biuret Test 4	Average % Biuret	STDEV
By 2003-14 Method								
25-012-05	Sulfur coated urea	39-0-0	0.911	0.943	0.979	0.933	0.942	0.025
25-068-06	Triazone Fertilizer	26-0-0	0.118	0.124	0.131	0.139	0.128	0.008
25-070-01	Fertilizer "A"	15-15-15	0.668	0.689	0.762	0.708	0.707	0.035
25-070-02	Fertilizer "B"	25-11-10	0.296	0.356	0.440	0.416	0.377	0.056
By 983.1 Method								
25-012-05	Sulfur coated urea	39-0-0	0.795	0.795	0.794	0.791	0.794	0.002
25-068-06	Triazone Fertilizer	26-0-0	5.793	5.854	5.856	5.814	5.829	0.027
25-070-01	Fertilizer "A"	15-15-15	0.560	0.560	0.557	0.555	0.558	0.002
25-070-02	Fertilizer "B"	25-11-10	0.299	0.302	0.303	0.300	0.301	0.002
By SRIC* Method (same as 2003-14)								
25-012-05	Sulfur coated urea	39-0-0	0.934	0.965	0.987	0.974	0.965	0.019
25-068-06	Triazone Fertilizer	26-0-0	0.120	0.131	0.130	0.134	0.129	0.005
25-070-01	Fertilizer "A"	15-15-15	0.663	0.711	0.734	0.727	0.709	0.028
25-070-02	Fertilizer "B"	25-11-10	0.286	0.367	0.388	0.382	0.356	0.041

* Shanghai Research Institute of Chemical Industry Testing Center

In another sets of tests, three of the commercial liquid fertilizers listed above, namely samples number 10, 11, and 12 were tested by three laboratories. Lab 1, and Lab 2 used the AOAC Official Method 2003.14, and Lab 3 used the AOAC 983.1 Official Method. Results are listed in Table 8.

Table8

Comparisons of results for sample #10, #11, and #12 by two HPLC Methods

Sample # 10 (28-0-0)	Sample # 11 (26-0-0)	Sample #12 (30-0-0)
% Free Urea		
(AOAC 2003.14)		**(AOAC 983.1)**
Lab 1	**Lab 2**	**Lab 3**
13.6	13.9	15.8
30.5	30.3	30.2
21.9	21.7	23.7

Results from Table 8 showed that AOAC Official Method 983.1 was not suitable for accurate analyses of this class of liquid fertilizer samples, due to incomplete separation of the urea peak from others.

Conclusions

The AOAC Official Methods 959.03 (Urease Method) and 983.01 (HPLC Method) are frequently inaccurate and therefore not suitable for measuring the amounts of free and unreacted urea in liquid urea-formaldehyde condensate products. AOAC Official Method 2003.14 consistently provides more accurate analytical results for measuring the contents of free urea in these classes of fertilizers.

Acknowledgements

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OPEN FOR COMMENTS

Proposed Modification to AOAC Official Method 959.03 Urea In Fertilizers

July 25, 2016: The AOAC Research Institute announces a notification of a proposed change in status of an AOAC Final Action Official Method 959.03 [Final Action 1960]. The open public comment period for the proposed modification of AOAC Official Method 959.03 will be posted for a minimum of 30 days. The comment period opens on **Monday, July 25, 2016** and closes **Wednesday, August 17, 2016.** Comments will be compiled, reviewed, and intended to obtain input on the proposed modification. The documents may be revised if necessary, based on comments received. Any interested party may submit comments.

A modification to AOAC Official Method 959.03: Urea in Fertilizers [Final Action 1960] is being proposed. Please see the following information regarding the modification:

Summary of Proposed Modification or Extension

By providing the statistical data, it will prove that the AOAC Official Method 959.03 is not suitable for urea-formaldehyde condensate fertilizer products and an editorial/clerical change is proposed.

Summary of how the proposed modification will be evaluated

By providing statistical data measuring the free urea contents in at least 8 different samples of these fertilizers by comparing the results of their free urea contents using both AOAC Official Method 959.03 (Urease Method) and AOAC Official Method 2003.14 Urea in Water-Soluble Urea-Formaldehyde Fertilizer Products and in Aqueous Urea Solutions [Final Action 2009].

Comment Process

AOAC requests that the following guidelines be observed in providing comments regarding the above modification:

1. Please specify if the comment is an editorial, content, or disagreement comment.
2. Provide rationale as to why the comments should be considered.
3. All comments are due within 30 days of the initial posting date (**deadline**: **Wednesday, August 17, 2016**). AOAC reserves the right to not to accept comments received after the deadline.
4. Editorial comments provide additional clarification or correct typographical errors. Please suggest alternative wording or typographical corrections.
5. Content-related comments provide technical clarity and comprehensiveness. Please suggest the appropriate technical language. Documents will be reviewed by AOAC for technical accuracy and clarity.
6. Disagreement comments reflect a perspective on content documented/undocumented in the drafts. Please provide language that document the perspective or position.

To provide comments and review the proposed modification of AOAC Official Method 959.03, please use the link at HTTPS://FORM.JOTFORM.COM/61666298869175.

Evaluation of the Determination of Free Urea in Water-Soluble Liquid Fertilizers containing Urea and Ureaforms by Urease Method and by HPLC Methods

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Abstract

Currently there are three AOAC Official Methods for the determination of urea in fertilizers.

AOAC Official Method 959.03, Urea in Fertilizers, Urease Method, First Action 1959, Final Action 1960. This method is based on the use of fresh commercial 1% urease solution, or preparation of such solution from urease powder in water, or from jack bean meal in water[1].

AOAC official Method 983.01, Urea and Methyleneureas (Water-Soluble) in Fertilizers, First Action 1983, Final Action 1984, is based on liquid chromatography with refractive index detector using water as mobile phase and an ODS column[1].

AOAC Official Method 2003-14, Determination of Urea in Water-Soluble Urea-Formaldehyde Fertilizer Products and in Aqueous Urea Solutions, First Action 2003, Final Action 2008, is also based on liquid chromatography with UV detector using 85%:15% Acetonitrile: Water as mobile phase and a propylamine column[2].

The Urea Method, AOAC Official 959.03 is very much dependent to the nature of the urease enzyme. The method was developed in 1960 and used for simple urea fertilizer solutions. With the advent of complex fertilizers compositions, especially with the class of liquid Triazone Fertilizers, and water-soluble ureaforms, the analyses of free urea in these fertilizers by the urease method is often inaccurate and inconsistent.

The AOAC Official Method 983.01 is not always reliable due to the interference of some of the components of these fertilizers, and due to the fact that the use of water as mobile phase does not always separate the free urea from other components[3].

The AOAC Official Method 2003.14 was subjected to Ring Test Studies and showed it could be used for the determination of "free urea" in these classes of fertilizers with good accuracy and precision[3].

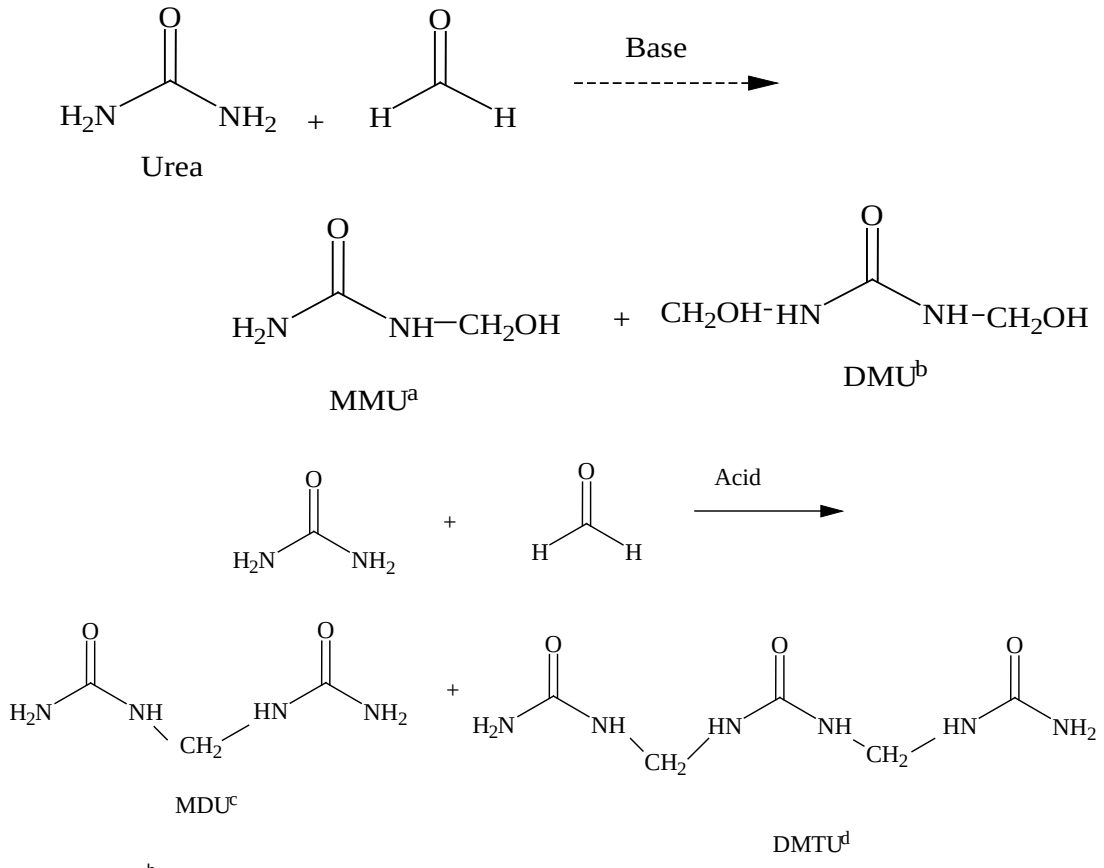
Introduction

The critical comparison of some currently manufactured commercial slow and controlled release nitrogen (SRN, and CRN)-containing fertilizers, especially liquid products, whether by means of technical

data sheets, product labels or otherwise, is unfortunately not always as an easy task for the potential user. In this matter, particular attention must be paid to the methods used by various manufacturers for the determination and reporting of the amount of SRN, and CRN present.

The SRN in most commercial liquid SRN-containing fertilizers presently manufacture in the United States, Canada, and Europe is comprised of the water-soluble reaction products of urea with formaldehyde, or from the reaction products of urea, formaldehyde and ammonia.

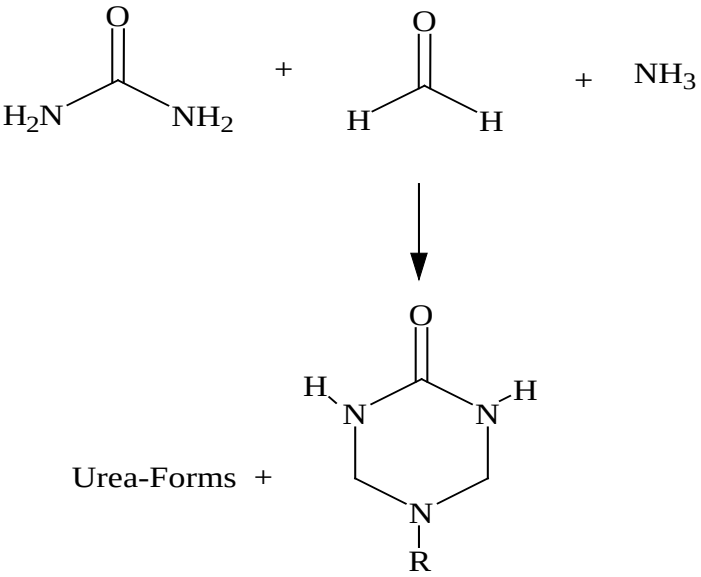
In the reaction of urea and formaldehyde, based on the nature of the catalysts, whether an acid catalyzed reaction, or a based catalyzed reaction, different urea-formaldehyde adducts (ureaforms) will form and the resulting product will be either liquid or solid[4].



[a] monomethylourea; [b]dimethylolurea;[c]methylenediurea; [d]dimethylenetriurea

Reaction of urea and formaldehyde is not 100% and some unreacted urea will remain in the final product. If the reaction product is a solid, the amount of unreacted and free urea could be determined by AOAC Official Method 945.01 (Water-Soluble Nitrogen), and AOAC Official Method 955.05 (Nitrogen Activity Index). Determination of water-insoluble nitrogen in mixed fertilizers has been studied by Katz, et.al.[5,6]

In the reaction of urea, formaldehyde, and ammonia, a highly water soluble ring structure known as urea-triazone predominantly forms.

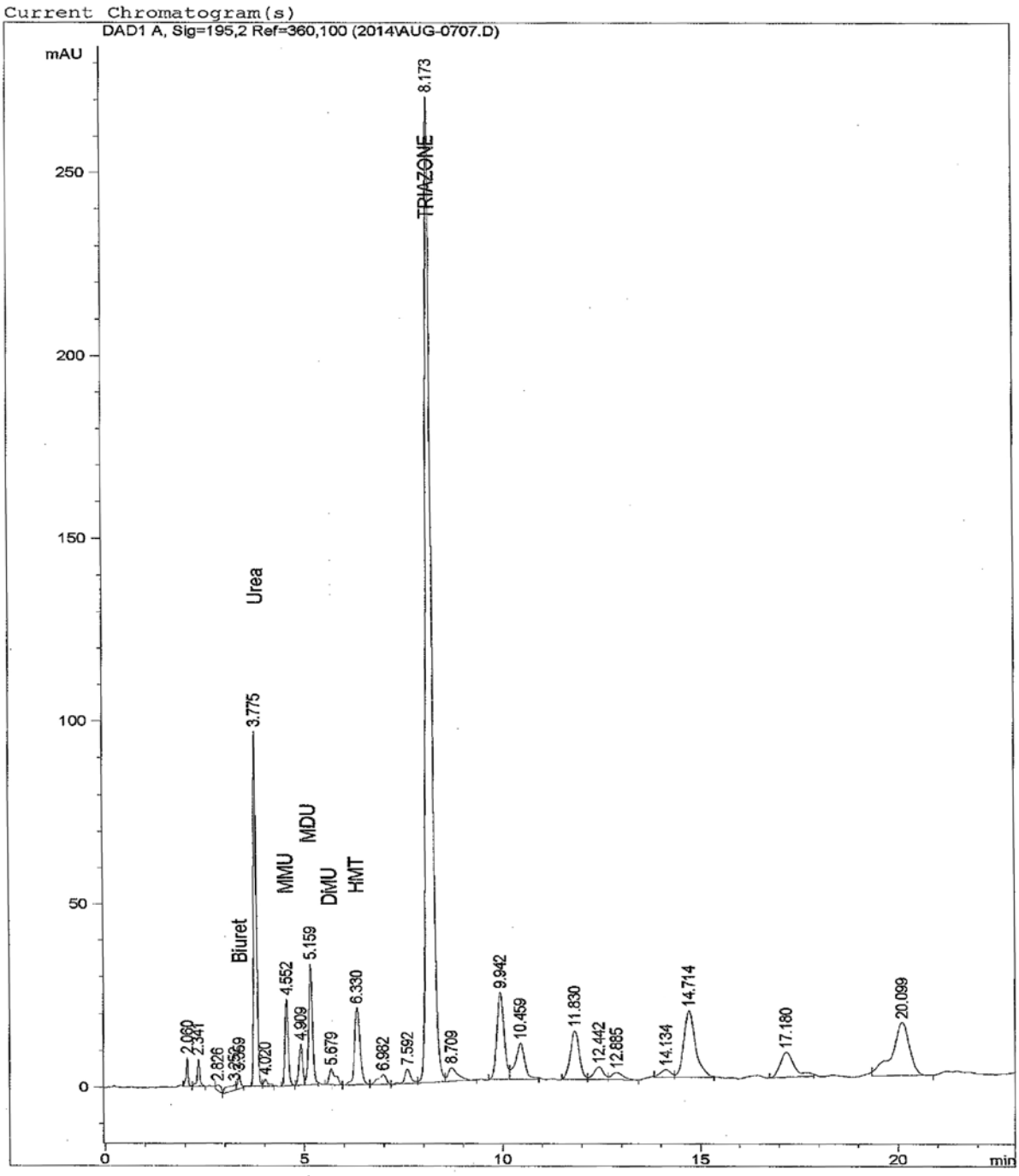


$R = H, CH_3, CH_2OH, CH_2NHCONH_2$

In most of these products produced from the reaction of urea and formaldehyde, or urea-formaldehyde-ammonia, the reaction products contain SRN in the form of aqueous solutions of water-soluble organic nitrogen species and some unreacted, free urea. The unreacted or free urea provides readily available nitrogen and therefore should be deducted from the slowly released nitrogen portion.

Quantitative analysis of these SRN fertilizer solutions for all of the reacted nitrogen forms in these fertilizers is time consuming and impractical or even impossible. The complex composition of an example of a fertilizer produced from the reaction of urea, formaldehyde, and ammonia is shown in Figure - 1 below:

Figure 1
Liquid Chromatography of Triazone Fertilizer



(MMU = monomethylolurea; MDU = methylenediurea; DMU = dimethylolurea; and HMT = hexamethylenetetramine)

Therefore, the direct determination of the amount of SRN by summingthe amounts of reacted organic N from various forms is not a viable option. Consequently, SRN content is most often determined as the difference of the unreacted free urea subtracted from total the N, both of which can be fairly easily determined. The remainder from this subtraction is the combined reacted organic N (or SRN), except in cases where some other nitrogen form such as nitrate may have been added after the reaction in order to make a mixed fertilizer.

Currently, there are three AOAC Official Methods available for the determination of the free and unreacted urea in liquid and water-soluble urea containing fertilizers.

The AOAC Official Method 959.03 is the most well-known and the oldest method for urea determination in fertilizers. This method, which was introduced in 1959, is based on the quantitative hydrolysis of urea to ammonia by means of urease enzyme. The amount of ammonia generated by hydrolysis is subsequently determined and reported as equivalent urea or urea N by a simple titration. With most simple and conventional (i.e. non-SRN-containing) liquid fertilizers, the urease method works very well. Urea-based methods are well accepted and usually quite suitable for urea determinations in most urea-containing fertilizers, however, they have been found to demonstrate significant problems, particularly with incomplete recovery of urea, when applied to such determinations in Urea-Formaldehyde (UF) fertilizer solutions. Comparisons of the HPLC methods, particularly the AOAC Official Method 2003.14 with results from the urease method, frequently produced results that were not in agreement with each other. Further study also showed that urea results from the urease methods often were not reproducible. Thus a controlled study, which is the principle subject of this article, was carried out to confirm and elaborate upon these observations, and to suggest some likely reasons for the reduced effectiveness of the urease method with UF fertilizers.

Experimental

Materials

Twelve of the most commonly available commercial liquid urea-based fertilizers were selected for the determination of their free urea contents using the three aforementioned methods. These commercial samples were:

1. LF3060 (30-0-0), Koch Agronomic Services, LLC, 4111 E. 37th St. N, Wichita, KS 67220, USA
2. CoRoN (28-0-0), Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017
3. Arclin 28-0-0, Arclin Resins, Research & Technology, 4754 28th St., Springfield, OR 97477
4. Greenfeed 27-0-0 (light Green liquid), Plant Food Company, 38 Hightstown-Cranbury Station Road, Cranbury, NJ 08512
5. Greenfeed 27-0-0 (Dark Green liquid), Plant Food Company, 38 Hightstown-Cranbury Station Road, Cranbury, NJ 08512
6. NDemand 30-0-0, Wilbur-Ellis Agribusiness, 3300 S. Parker Road, Suite 500, Aurora, CO 80014, USA
7. NDemand 30L, Wilbur-Ellis Agribusiness, 3300 S. Parker Road, Suite 500, Aurora, CO 80014, USA
8. Gradual N (30-0-0), Winfield Solutions, LLC, 1080 County Road West, Shoreview, MN 55126
9. N-28 clear Fertilizer (28-0-0), Advachem, Route de Wallonie, Darse d'Hautrage, BE 7334 Hautrage
10. N-Sure (28-0-0), Tessenderlo Kerley, Inc., 2255 N. 44th St., Suite 300, Phoenix, AZ
11. Trisert NB (26-0-0), Tessenderlo Kerley, Inc., 2255 N. 44th St., Suite 300, Phoenix, AZ
12. Formolene Plus (30-0-0), Tessenderlo Kerley, Inc., 2255 N. 44th St., Suite 300, Phoenix, AZ

These fertilizers are liquid and were used without any further modifications, unless specified in the method, (i.e., dilution).

The abovetwelve commercially available U-F liquid fertilizers were tested by the authors' lab (Lab 1) and by an independent lab (Lab 2) using AOAC Official Method 2003.14 for the determination of their unreacted (free) urea by HPLC. Each sample was analyzed for its free urea content in duplicate. The inter laboratory results were compared to each other for the consistency of this method for the determination of free urea contents in these fertilizers by comparing each value obtained from the means of duplicate analyses. Analytical results, means and standard deviations are shown in Table 2.

The same materials were also analyzed by a commercial lab using the AOAC Official Method 953.01 (Urease Method) for the determination of their free urea contents. Each sample was analyzed in duplicate and their means were used for comparisons with the means of those results obtained by Lab 1, and Lab 2 using the AOAC Official method 2003.14. The commercial lab used powdered form of urease made from Jack bean meal.

In addition, different sources and forms of urease enzyme were tested to understand what effects (if any) of the urease enzyme has on the determination of free urea contents of this class of fertilizers.

Analytical methods

AOAC Official Method 2003.14[2]

A. *Principle*

A precisely weighed portion of homogenous urea or homogenous water-soluble urea-containing liquid UF fertilizer sample was diluted to volume with solvent of the same composition used as the liquid chromatographic mobile phase. Ureawas determined via high performance liquid chromatography (HPLC) employing ultraviolet wavelength detection. The area beneath the absorption peak due to urea in the sample was compared with the area beneath the absorption peak for urea in an external standard solution prepared with pure urea in the mobile phase solvent.

B. *Apparatus*

(a) *Liquid Chromatograph.* – Requires a high performance liquid chromatograph capable of isocratic delivery of mobile phase at 2 ml/min at 204 bars (3000 psig) and having a UV absorption detector capable of stable operation at 195 nm (Acetonitrile and water absorption cutoff). Instrument operating conditions are listed in Table 1:

Table - 1
HPLC Operating Conditions and Settings

Operating conditions	Setting
Flow Rate	1.3ml/min
Mobile Phase Temperature	Ambient
Column temperature	35 ^{0}C
Detector Wavelength	195nm
Injection Volume	10 µl

For best precision a fixed volume sample loop is preferred to syringe injection of samples and standards. To analyze fertilizer solution for urea, allocate 14 minutes for each injection, 12 minutes for run time and 2 minutes for post run time. For more complex fertilizer solutions, allocate a total of 43 minutes for each injection, which includes 23 min of run time and 20 min of post-run timeto avoid overlapping.

(b) *HPLC Column* – The chromatography column is a 4.6 mm ID x 250 mm length amino propyl (NH_2) column, 5µ particle size. Examples are Phenomenex amino propyl column Spherex, Part # 00G-00051-E0, or Thermo Scientific Hypersil APS-2 column, Part # 30703-254630. Before use, new columns must be conditioned as described in Section (c) hereof.

(c) If the HPLC column is new or has not been in service for about a week or more, it must be conditioned as follows before using. A conditioned column will usually last for about a year depending on the number of analyses. This conditioning step is only applied to new columns or columns that are not used on a weekly basis:

(1) Using the HPLC instrument, wash the column for about two to four hours at room temperature with HPLC-grade isopropanol at a flow rate which will maintain at least 200 bars column back pressure (typically about 1 ml/min).
(2) Follow step 1 with a second column wash with 100% HPLC-grade acetonitrile at room temperature for 4 hours.
(3) Follow step 2 with a final column wash using mobile phase solution [see C(a)] at 1.3 ml/min and normal analytical operating conditions. This wash should continue for 2 hours or as long thereafter as needed to obtain a stable base line.

C. *Reagents*

(a) *Mobile Phase* – 85% (v/v) acetonitrile with water. Use LC grade acetonitrile having 190 nm maximum UV cutoff and LC grade water.
(b) *Urea standard-ACS reagent, 99-100.5%, Sigma-Aldrich Chemical Company, Milwaukee, WI.*

D. External Standard

Urea Standard - Accurately weigh amounts of 100% pure urea of approximately 0.0150g and 0.0300g into separate 100 ml volumetric flasks. Use ultrasound for three minutes to dissolve and then dilute to volume with mobile phase solution. Shake well.

E. Sample Preparation

Accurately weigh a portion of uniform sample containing an estimated amount of free urea between 0.0150g and 0.0300g into a 100 ml volumetric flask. Use ultrasound for three minutes to dissolve and then dilute to volume with mobile phase solution and shake well to assure homogeneity. (Note: For solid samples or fluids containing substantial undissolved solids, ultrasound for 5 minutes shake periodically over a period of about 15 minutes to ensure that all urea has an opportunity to dissolve.) Filter a portion through a 0.45 µm porosity (or finer) filter before injecting onto HPLC column. Samples should be analyzed on the same day as prepared.

Determination

(a) Inject 10 µl of each urea standard until two consecutive injections of each give the same peak area within ± 2% for the same standard. Average the peak areas for the accepted standard determinations.

(b) Inject 10 µl of prepared sample. Identify the urea peak by retention time relative to a urea standard and note if the peak area falls within the range of the high and low standards. If not, prepare a new sample with the weight adjusted to permit peak to fall within the standard range.

(c) Perform sufficient sample injections (minimum of two) from the same sample flask such that at least two consecutive determinations yield peak areas which agree with each other to a precision of at least ± 2%.[2] Determine the average value of agreeing peak areas.

Calculations

Calculate the average instrument urea working standard response from n standards as:

$$\text{Urea Factor} = \left[\sum \left(\frac{AP_r}{W_r}\right)\right] / n$$

Where AP is the average response of 2 or more agreeing peak area for the working standard r, W_r is the weight of urea in the 100 ml working standard and n is the number of agreeing working standards used in the calculation.

AOAC Official Method 959.03[1]

Reagents

Either fresh commercial 1% urease solution was used or the urease solution was prepared by dissolving 1 gram of urease powder in 100 ml of distilled water, or one gram jack bean meal was transferred into 100 ml of distilled water and shaken for 5 minutes. Ten ml of this solution was transferred into a 250 ml Erlenmeyer flask, and diluted with 50 ml distilled water. The enzyme activity was determined by titration with 0.1 N HCl in the presence of methyl purple (Fisher Scientific, Suwanee, GA) to a reddish purple color, then back titrated with 0.1N NaOH to a green color. The amount of 0.1 N hydrochloric acid required to neutralize the remainder of solution was calculated and added to this solution. The enzyme

activity was verified periodically, and any source which did not produce solution capable of hydrolyzing 0.1 g urea/20 mL solution was discarded.

Determination

Ten grams of each liquid ureaformaldehyde fertilizer sample (containing ≤1.0g of urea) was transferred to a 15 cm Whatman No. 12 fluted filter paper (Fisher Scientific). This was leached with approximately 300 ml of de-ionized water (D-H_2O) into a 500 ml volumetric flask. Next, 75-100 ml of saturated barium hydroxide, $Ba(OH)_2$(Fisher Scientific) was added to precipitate out any phosphate present. Then, 20 ml of 10% sodium carbonate, Na_2CO_3(Fisher Scientific) solution was added to precipitate any excess Ba. This was diluted to volume, mixed, and filtered through a 15 cm Whatman No. 12 fluted filter paper. Then, 50 ml of the filtrate was transferred to a 250 ml Erlenmeyer flask, and 2-4 drops of methyl purple was added followed by addition of 2N HCl to form a reddish purple color (acidic). This was neutralized with 0.1 N NaOH to a green color. Finally, 20 ml of neutral urease solution was added and the flask closed with a rubber stopper. After one hour storage at room temperature, the flask was cooled in an ice-water bath and its content was titrated with 0.1N HClto a full purple color, and then a 5ml excess of 0.1 N HCl was added. Excess HCl was back titrated with 0.1 N NaOH to a neutral end point (green color).

Percent urea calculated as follow:

$$\%\ Urea = \frac{[ml\ 0.1\ N\ HCl - ml\ 0.1\ N\ NaOH)x\ 0.3003^{*}]}{g\ sample}$$

*0.3003 = this factor takes into account the molecular weight of urea, the conversion of the milliequivalent result of V x N, and the conversion to %.

Results and Discussion

The free urea contents of each fertilizer sample were measured in duplicates by the AOAC Official Method 2003.14 by Lab 1 and Lab 2.

The results of analyses and t-test calculation of the twelve commercial liquid fertilizer sampleslisted above from Lab 1 and Lab 2 by the AOAC Official Method 2003.14 are listed in Table 2.

Table – 2
Analyses of Twelve Fertilizer Samples for Urea by AOAC Method 2003.14

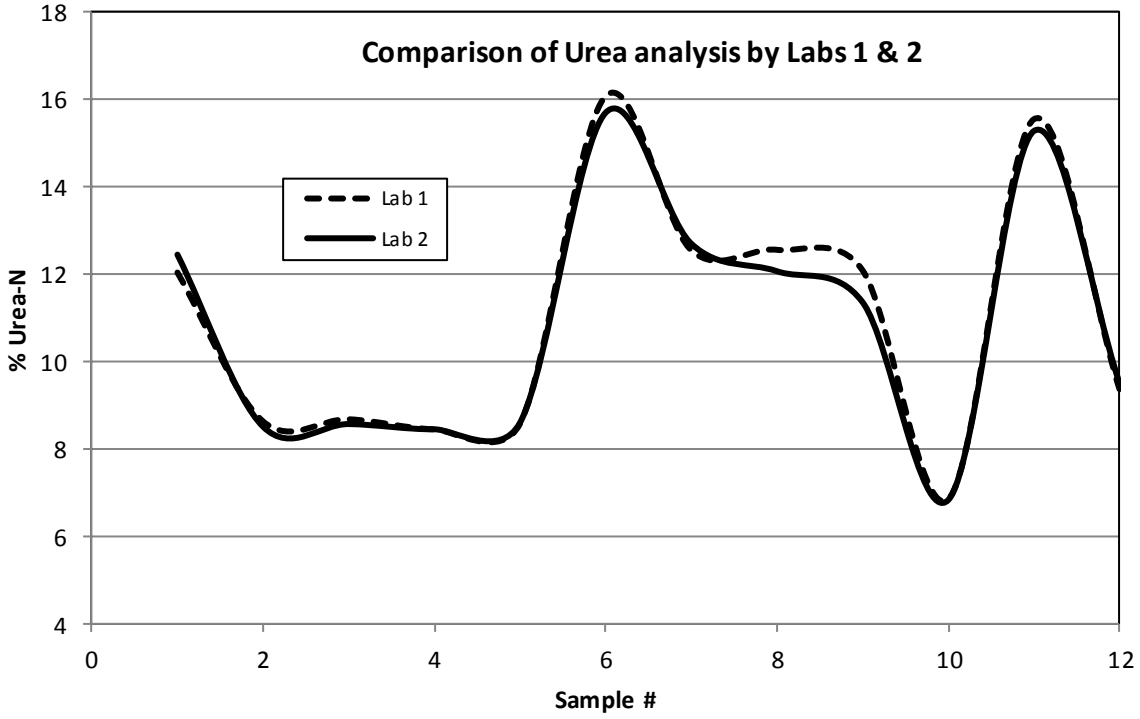
Fertilizer Sample	Lab ID	Total –N*	% Urea-N		Mean	Std. dev.
			Lab 1	Lab 2		
1	26-068-03	29.89	12.04	12.45	12.25	**0.21**
2	26-051-01	28.54	8.63	8.51	8.57	**0.06**
3	26-068-02	28.44	8.68	8.57	8.63	**0.05**
4	26-076-07	28.33	8.45	8.45	8.45	**0.00**
5	26-076-06	28.47	8.58	8.60	8.59	**0.01**
6	26-076-08	24.95	16.08	15.70	15.89	**0.19**
7	26-079-01	30.78	12.55	12.70	12.63	**0.07**
8	26-086-04	30.06	12.56	12.07	12.32	**0.25**
9	26-103-02	28.01	12.09	11.36	11.73	**0.37**
10	26-103-03	28.13	6.85	6.83	6.84	**0.01**
11	26-100-03	26.36	15.55	15.28	15.42	**0.14**
12	26-010-02	29.87	9.37	9.51	9.44	**0.07**
Average		$\bar{X}$	10.95	10.84	-	**-**
Standard Deviation		σ_x	2.97	2.87	-	**-**
Number of samples		n	12	12	-	**-**
Variance		σ^2	8.80	8.21	-	**-**
Pearson Correlation		0.99	-	-	-	**-**
T Stat		1.30	-	-	-	**-**
P(T<t) one-tail		0.11	-	-	-	**-**
t Critical one-tail		1.80	-	-	-	**-**
P(T<=t) two-tail		0.22	-	-	-	**-**
T Critical two-tail		2.20	-	-	-	**-**

* Note: Total-N includes other forms of nitrogen in addition to unreacted (free) urea

A statistical comparison (Students t-test, df, and, P) indicated that the results from two labs were statistically similar.

The results for the analyses of the Free and Unreacted urea in these twelve fertilizers by two independent Labs using AOAC Official Method 2003.14 are shown graphically as well in Figure 2.

Figure 2
Schematically comparisons of HPLC Results by two Labs



When the same samples were analyzed by urease method based on the AOAC Official Method 959.03,the results are much different and in this case showed a high biased in ten of the analyses and low biased in the remaining two analyses. The results and the differences between the results from the urease method and the HPLC method are shown in Table 3.The lowest % Urea-N difference from the two methods shown in Table 3is -0.45 and the highest % Urea-N difference is 2.42. These results were obtained by a commercial lab using powdered form of urease made from Jack Bean meal.

Table-3
Urease Analyses of Twelve Fertilizer Samples & Comparisons with the HPLC Results for Urea

		% Urea-N	%Urea-N	% Urea-N
Total-N	**Fertilizer Sample**	**Urease Method AOAC 959.03**	**HPLC Method AOAC 2003.14 (Means from two Labs)**	**Difference Urease-HPLC (Mean)**
29.89	**1**	13.26	12.25	***+1.02***
28.54	**2**	9.87	8.57	***+1.30***
28.44	**3**	10.06	8.63	***+1.44***
28.33	**4**	9.33	8.45	***+0.88***
28.47	**5**	9.91	8.59	***+1.32***
24.95	**6**	15.44	15.89	***-0.45***
30.78	**7**	14.81	12.63	***+2.19***
30.06	**8**	14.08	12.32	***+1.77***
28.01	**9**	8.89	11.37	***-2.48***
28.13	**10**	8.94	6.84	***+2.10***
26.36	**11**	16.18	15.42	***+0.77***
29.87	**12**	11.86	9.44	***+2.42***

In another series of studies supplies of seven different analytical quality urease enzyme sources were obtained from five well-known suppliers of laboratory chemicals which are identified as numbers 1 through 7. It is highly probable that one or more of these common sources are found in a large number of fertilizer analytical laboratories. Represented are four brands of urease powder, one brand of urease tablets and two brands of urease-glycerol extract solution. Only one supplier provided the information regarding the urease activity as part of its product label.

Tests using the urease enzymes in this study were carried out in accordance with the AOAC Official Method 959.03, with one exception where anaccommodation for the use of urease tablets or glycerol extract was required. The AOAC urease method specifies the use of a 1% aqueous solution of urease powder or Jack bean meal.

According to the AOAC urease method, 20ml of 1% solution of urease should be used and also the urease activity should be such that it will hydrolyze at least 0.1 gram of urea under the conditions specified in the method. The weight of fertilizer sample should also be such that the amount of urea in the final working volume does not exceed 0.1 gram.

In one series of tests, pure urea (99.4%) was used. The weights of urea samples used were 0.1g, 0.3g, 0.6 g, and 1.0 gram. These urea samples were analyzed using seven ureasematerials from different sources. The degrees of urea hydrolysis with the seven different urease materials are shown in Table 4.

Table-4
Analyses of Urea using Different Sources of Urease

Urease Source	Urease Amount	% Hydrolysis of urea from the amount used			
		0.1 g	0.3 g	0.6 g	1.0 g
1	20 ml (1% Sol.)	100.1	100.1	99.7	99.9
2	10 ml Glycerol Ext.	99.9	99.7	99.7	69.9
2	20 ml Glycerol Ext.	99.9	99.7	99.7	99.4
3	20 ml (1% Sol.)	99.6	99.7	97.6	62.7
4	20 ml (1% Sol.)	99.3	99.7	83.2	52.6
5	2 tablets	100	98.6	69.3	45.5
6	20 ml Glycerol Ext.	99.9	99.9	99.7	99.5
7	20 ml (1% Sol.)	100.3	100.1	99.9	99.6

In the amount used, all seven urease sourceshydrolyzed up to about 0.3 grams, thus exceeding the AOAC method requirements. However the urease tablets (source No. 5) begins to fade in effectiveness at 0.3 grams urea, with only 98.6% hydrolyzed, and two urease powders (No. 3and No. 4) begin to fail at about 0.6 grams of urea. The two other powder samples (No. 1 and No. 7) and the two urease glycerol extracts (No. 2 and No. 6) in 20 ml increments all hydrolyzed up to 1.0 gram of urea. Powder No.7 was known from the manufacturer's label to have a very high urease activity. From the results, it appears that sources 1, 2, and 6 were high activity urease samples. These data show that the AOAC Official Method 959.03 works well for pure urea when applied within the limits of the method. However, if the amount of urea exceeds the recommended amount in the method, there are inconsistencies with the results and some urease sources work better than others.

The inconsistencies with the Urease Method are obvious when the method applies to the more complex fertilizer samples, namely, the Urea-Triazone liquid solution containing mixed compositions of urea-forms and triazone moieties (Table 2).

More tests were done to further illustrate these inconsistencies even further.

In the following sets of experiments, five Urea-Triazone fertilizers from four different manufacturers were analyzed for their claim of %SRN. The percentage of free and unreacted urea in these samples was analyzed by the HPLC and by the urease methodusing powdered urease enzyme from Jack bean meal. The results are shown in Table 5.

Table 5
Comparisons of the Results by HPLC (2003-14) and by Urease Method(Powdered Sample)

Product % Total N	%SRN Claimed	SRN Determination	
		Method	
		HPLC (2003-14)	Urease
28-0-0	**72**	72	88
30-0-0	**60**	60	77
30-0-0	**50**	50	68
28-0-0	**75**	50	70
30-0-0	**85**	46	80

The results by the urease method for the SRN contents (i.e., Total N minus Free Urea-N) of these known fertilizers were always different from the claimed amount, while the results by HPLC were on target for three of five samples and off for two samples.

The following results further illustrate the inconsistencies of the Urease Method when applied to this class of fertilizers[7]. Comparisons of the urea results obtained with the different urease sources listed in Table 4 with the HPLC Method 2003.14 are shown in Table6. The results are for four different aqueous Urea-Triazone fertilizers and are shown in three ways, (1) as the absolute weight of urea found in each sample volume by both methods, (2) as the weight percent urea in the fertilizers, and (3) as the percent recovery of urea by the Urease Method relative to 100% recovery by the HPLC Method.

These results clearly show that the Urease Method using urease sources 1, 3 and 4 finds less urea in all four fertilizer samples in comparisons with the results from the HPLC Method. The differences are substantial. For example, sometimes less than half as much urea is recovered by the Urease Method. For the source No. 2, it makes a significant difference whether 10 ml of Glycerol extract or 20 ml of glycerol extract was used. Source No. 5 (Urease tablets) showed the poorest recovery results. All of these urease sources should normally be expected to totally hydrolyze the urea in these fertilizers. However, four of these Urease sources (Nos. 1, 3, 4, and 5) plus 10 ml of the source No.2 Glycerol extract significantly failed to hydrolyze all of the urea in any of these four fertilizer samples.

Table-6
AOAC 959.03 (Urease Method) vs. AOAC 2003.14 (HPLC) Results for Analyses of Unreacted Urea in Urea-Triazone Liquid Fertilizers

amount used	Sample	Urease	HPLC	Urease	HPLC	Urease vs HPLC
1	A	0.20	0.34	20.2	33.7	60
20ml 1% Solution	B	0.11	0.16	10.9	16.1	68
	C	0.24	0.32	23.5	32.1	73
	D	0.27	0.35	27.3	35.1	78
2	A	0.17	0.34	16.9	33.7	50
10ml Glycerol Ext.	B	0.08	0.16	7.5	16.1	47
	C	0.14	0.32	14.2	32.1	44
	D	0.25	0.35	25.4	35.1	72
2	A	0.34	0.34	34.3	33.7	102
20ml Glycerol Ext.	B	0.17	0.16	17.0	16.1	106
	C	0.32	0.32	32.4	32.1	101
	D	0.33	0.35	33.4	35.1	95
3	A	0.15	0.34	15.0	33.7	45
20ml 1% Solution	B	0.10	0.16	9.5	16.1	59
	C	0.18	0.32	17.8	32.1	55
	D	0.32	0.35	32.4	35.1	92
4	A	0.18	0.34	18.1	33.7	54
20ml 1% Solution	B	0.11	0.16	11.0	16.1	68
	C	0.18	0.32	18.4	32.1	57
	D	0.24	0.35	23.5	35.1	67
5	A	0.17	0.16	6.9	16.1	43
Two Tablets	B	0.13	0.32	13.0	32.1	40
6	B	0.17	0.16	16.9	16.1	105
10 ml Glycerol Ext.	C	0.33	0.32	33.1	32.1	103
6	B	0.17	0.16	17.1	16.1	106
20 ml Glycerol Ext.	C	0.33	0.32	33.2	32.1	103
7	A	0.34	0.34	34.0	33.7	101
20ml 1% Solution	B	0.16	0.16	16.2	16.1	101
	C	0.32	0.32	32.3	32.1	101
	D	0.33	0.35	33.0	35.1	94

In quantitative terms, hydrolyses of urea in these four fertilizer samples by these urease sources tended to be in the range of 40-80%. Each of the urease sources should have hydrolyzed all the urea in these type of fertilizers based on the tests done using the pure urea (Table 4), within the scope of the Urease Method. Fertilizer “B” contains only about half of the urea of the other three fertilizer samples (A, C, and

D). However, even with less free, unreacted urea in the sample, recovery by these urease sources was not improved. From the data in Table 5, the lower activity urease sources failed substantially to hydrolyze all the urea in these UF fertilizers, while the high activity sources did hydrolyze all the urea. The fact that high activity sources (No. 1, 7, and 20 ml extracts of No.2) show the same percent urea hydrolyses as the amount found by HPLC analyses, support that such percentages urea are indeed present in these UF fertilizers samples. The combined performances of HPLC and the high activity urease sources indicate that the AOAC Method 959.03 is very much dependent on the type of urease.

The above data show that analyses of Urea-Formaldehyde fertilizer solutions using the urease method are not consistent. In most cases, the amount of free and unreacted urea analyzed by the urease method showed a low biased (Table 6). However, in some occasions the results showed a high bias (Table 3).

These data support the discussion that AOAC official Method 959.03 (Urease Method) is not suitable for the determination of free-unreacted urea in the Urea-Formaldehyde fertilizer solutions. The HPLC Method consistently provides more accurate analyses of unreacted urea in this class of fertilizers.

As mentioned above, there are two AOAC Official Methods by HPLC for the determination of unreacted urea in these fertilizers, namely AOAC Official Method 983.01, and AOAC Official Method 2003.14.

The advantage with AOAC 983.01 is the use of water as the mobile phase, however due to the use of water, the peak separations in the complex compositions of these fertilizers (Figure 1) is not efficient and some of the peaks might overlap with that of urea resulting in erroneous data. A collaborative study[3] by the International Standards organization (ISO-CD 18643) involving 13 laboratories for the biuret content of several fertilizers, including the water-soluble Urea Triazone fertilizers,has showed that AOAC 983.01 HPLC method results in erroneous analyses of some of these UF fertilizer solutions. The same study showed that the AOAC 2003.14 method accurately predicts the amounts ofunreacted urea in these fertilizers.

Table 8 shows the inconsistency of the AOAC Official Method 983.01 (HPLC) for the determination of biuret in the Urea-Triazone fertilizers (Grade 26-0-0). The biuret co-elutes with the free and unreacted urea in these fertilizers and their peaks overlap resulting in erroneous analytical data.

Table 7

Comparisons of the analytical results for Grade 26-0-0 by AOAC 983.1 & AOA 2003.14

Sample ID	*Samples Analyzed by Lab-1*	Grade	% Biuret Test 1	% Biuret Test 2	% Biuret Test 3	% Biuret Test 4	Average % Biuret	STDEV
By 2003-14 Method								
25-012-05	Sulfur coated urea	39-0-0	0.911	0.943	0.979	0.933	0.942	0.025
25-068-06	Triazone Fertilizer	26-0-0	0.118	0.124	0.131	0.139	0.128	0.008
25-070-01	Fertilizer "A"	15-15-15	0.668	0.689	0.762	0.708	0.707	0.035
25-070-02	Fertilizer "B"	25-11-10	0.296	0.356	0.440	0.416	0.377	0.056
By 983.1 Method								
25-012-05	Sulfur coated urea	39-0-0	0.795	0.795	0.794	0.791	0.794	0.002
25-068-06	Triazone Fertilizer	26-0-0	5.793	5.854	5.856	5.814	5.829	0.027
25-070-01	Fertilizer "A"	15-15-15	0.560	0.560	0.557	0.555	0.558	0.002
25-070-02	Fertilizer "B"	25-11-10	0.299	0.302	0.303	0.300	0.301	0.002
By SRIC* Method (same as 2003-14)								
25-012-05	Sulfur coated urea	39-0-0	0.934	0.965	0.987	0.974	0.965	0.019
25-068-06	Triazone Fertilizer	26-0-0	0.120	0.131	0.130	0.134	0.129	0.005
25-070-01	Fertilizer "A"	15-15-15	0.663	0.711	0.734	0.727	0.709	0.028
25-070-02	Fertilizer "B"	25-11-10	0.286	0.367	0.388	0.382	0.356	0.041

* Shanghai Research Institute of Chemical Industry Testing Center

In another sets of tests, three of the commercial liquid fertilizers listed above, namely samples number 10, 11, and 12 were tested by three laboratories. Lab 1, and Lab 2 used the AOAC Official Method 2003.14, and Lab 3 used the AOAC 983.1 Official Method. Results are listed in Table 8.

Table8

Comparisons of results for sample #10, #11, and #12 by two HPLC Methods

Sample # 10 (28-0-0)	Sample # 11 (26-0-0)	Sample #12 (30-0-0)
% Free Urea		
(AOAC 2003.14)		**(AOAC 983.1)**
Lab 1	**Lab 2**	**Lab 3**
13.6	13.9	15.8
30.5	30.3	30.2
21.9	21.7	23.7

Results from Table 8 showed that AOAC Official Method 983.1 was not suitable for accurate analyses of this class of liquid fertilizer samples, due to incomplete separation of the urea peak from others.

Conclusions

The AOAC Official Methods 959.03 (Urease Method) and 983.01 (HPLC Method) are frequently inaccurate and therefore not suitable for measuring the amounts of free and unreacted urea in liquid urea-formaldehyde condensate products. AOAC Official Method 2003.14 consistently provides more accurate analytical results for measuring the contents of free urea in these classes of fertilizers.

Acknowledgements

We give special thanks to Hugh Rodriguez, Thornton Laboratory, Tampa, FL, and John Hartshorn, Morral Chemical Co., Morral, OH, for collaborative support of this study. We are also indebted to Nancy Thiex and James Bartos for many helpful suggestions.

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7. In part with Late Thomas M. Parham, Jr., R&D Department, Tessenderlo Kerley, Inc., Phoenix, Arizona

AOAC RESEARCH INSTITUTE
AOAC *OFFICIAL METHODS OF ANALYSIS* (OMA)

OMAMAN-28: Simultaneous Determination of Arsenic, Cadmium, Calcium, Chromium, Cobalt, Copper, Iron, Lead, Magnesium, Manganeses, Molybdenum, Nickel, Selenium, and Zinc in Fertilizers by Microwave Acid Digestion and Inductively Coupled Plasma-Optical Emission Spectrometry Detection: Single Laboratory Validation

TECHNICAL EVALUATION CRITERIA	
Is the test kit method scientifically and technically sound?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Have sufficient controls been used, including those required to calculate the rate of false-positive and false-negative results where appropriate?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Is sufficient information included for system suitability determination and product performance or acceptance testing?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Are the conclusions statements valid based upon data presented?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Do you agree that the evidence or data from this and previous studies support the proposed applicability statement?	
ER 1	Yes
ER 2	Yes
ER 3	Yes

ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Are there sufficient data points per product evaluated in accordance with AOAC requirements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
General Comments about the Method Scope/Applicability:	
ER 1	Follows good Inorganic protocols. Similar to both EPA and ASTM methodology.
ER 2	Excellent work! This method will enable state fertilizer regulatory laboratories to simultaneously analyze nutrient guarantees and evaluate fertilizer metal levels in support of AAPFCO SUIP #25 requirements. The ability to obtain these results with a single sample preparation and analysis for a broad scope of commercial fertilizer product samples is invaluable. Our laboratory intends to adopt this methodology for the analysis of official fertilizer samples in support of Chapter 576, F.S.
ER 3	A multitude of elements (both hazardous and nutritive) are analyzed in a single digestate of fertilizer materials. Wonder if it would be possible to include S in the scope of the method for interlaboratory evaluation.
ER 4	Very good method verification. Draws attention to watchouts such as wavelength interferences.
ER 5	The addition of Group B metals is a reasonable modification to AOAC 2006.03. This method is needed to replace AOAC 965.09.
ER 6	Scope and applicability for this method appears suitable for mentioned metals.
ER 7	The method is applicable to a broad range of fertilizers.
ER 1	Basic Methodology
ER 2	The high level of detail provided in the digestion and detection sections is extremely helpful and will assist laboratories in performing the method properly. Method QC is very strong. Tables 6 and 7 and the Experimental Validation (method ruggedness tests) section are excellent. Finally, the results provided for the Magruder 2009-06 and NIST SRM695 demonstrate the effectiveness of this methodology.
ER 3	1. A multitude of elements (both hazardous and nutritive) analyzed in a single digestate greatly improves laboratory efficiencty. 2. Good details and creating calibration standards and results of calibration curves.
ER 4	Run sequence defined well to maintain calibration integrity.
ER 5	The manuscript is well written. The conclusions are supported by data within the manuscript.
ER 6	The manuscript explains well the scope of the method, the procedure and analytical results.

ER 7	Usable for a number of different ICP-OES models
Cons/Weaknesses of the Manuscript:	
ER 1	Address the importance of the sample prep reagents, especially for the Heavy Metals.
ER 2	None
ER 3	1. The unit in Table 9 for LOD and LOQ is incorrectly showing mg/L when it should be mg/kg. These are detection limits in the fertilizer in mg/kg after taking into account factors with digestate of 100 mL and sample wt of 1 g. They are not detection limits in the digestate which the mg/L units imply. 2. The instrument LOD and LOQ for Ni, Pb, and Se appear much too low. Are these possibly detection limits in the digestate at mg/L before calculated detection limits in the fertilizer in mg/kg? 3. A reference is presented on how LOD and LOQ were determined with a reference to (8). However, the reference list only goes up to (6). 4. Probably not necessary, but some interpretation and discussion of results for accuracy, precision, and comparability would have been nice.
ER 4	Perhaps state this is intended for total metal analyses and not for water soluble.
ER 5	Error in section G.(7). 400 mg/kg should be changed to 400 mg/L. 1 L acid-washed flask should be changed to 500 mL acid washed flask.
ER 6	This method could not be applicable for all possible fertilizers and metals (example: selenium).
ER 7	Not all of the metals are equally responsive to this method.
Yes	Address the importance of the sample prep reagents, especially for the Heavy Metals.
Supporting Data and Information: Does data from collaborative study support the method as written?	
ER 1	Yes
ER 2	Yes (SLV)
ER 3	yes
ER 4	Yes
ER 5	Yes
ER 6	Yes.
ER 7	Yes, it appears to.
Supporting Data and information: Does data collected support the criteria given in the collaborative study protocol?	
ER 1	Yes
ER 2	Yes
ER 3	yes
ER 4	Yes
ER 5	Yes
ER 6	Yes.
ER 7	Yes, it appears to.
Are there any concerns regarding the safety of the method?	
ER 1	No
ER 2	No, applicable safety information is included.
ER 3	no
ER 4	No

ER 5	No
ER 6	No.
ER 7	No
Are there any concerns regarding the data manipulation, data tables, or statistical analysis?	
ER 1	No
ER 2	No
ER 3	no
ER 4	No
ER 5	I have not seen the review provided by the Statistical Advisor.
ER 6	No.
ER 7	No

EDITORIAL EVALUATION CRITERIA Is the Validation Study Manuscript in a format acceptable to AOAC?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Is the method described in sufficient detail so that it is relatively easy to understand, including equations and procedures for calculation of results (are all terms explained)?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Are the figures and tables sufficiently explanatory without the need to refer to the text?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Are all the figures and tables pertinent?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Could some be omitted and covered by a simple statement?	
ER 1	No
ER 2	No
ER 3	No
ER 4	No
ER 5	No

ER 6	No
ER 7	No

Are the references complete and correctly annotated?	
ER 1	Yes
ER 2	Yes
ER 3	No, Only 6 references shown in reference list, but LOD section makes a reference to #8 reference.
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
Does the method contain adequate safety precaution reference and/or statements?	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes

«Project_Number»: «Company_Name»«Method_Name»

RECOMMENDATION: **Do you recommend that the ERP adopt this method as an AOAC *Official Method of Analysis (First Action status)*?**	
ER 1	Yes
ER 2	Yes
ER 3	Yes
ER 4	Yes
ER 5	Yes
ER 6	Yes
ER 7	Yes
AFTER FIRST ACTION STATUS: **Is there any additional information that the ERP should consider in order to recommend the method for Final Action status?**	
ER 1	No
ER 2	No
ER 3	No
ER 4	No
ER 5	No
ER 6	No
ER 7	No

Reviewers

ER 1	Dion Tsourides
ER 2	Patricia Lucas
ER 3	Frank J Sikora
ER 4	Jack Schmansky
ER 5	Scott Sabel
ER 6	Salvatore Parisi
ER 7	Keith Wegner

EXPERT REVIEW PANEL (ERP) FOR FERTILIZERS

EXPERT REVIEW PANEL FOR FERTILIZERS - UREA

Is this method recognized or adopted by another agency outside of AOAC?

1) Not at my knowledge
2) N/A
3) As an AOAC fertilizer method, this method is recognized and could be utilized by our laboratory for fertilizer sample analysis.
4) Yes

Does your organization support the proposed modification being submitted to AOAC? Please indicate explanations.

1) Yes, my organisation supports the change
2) Yes, many of our formulas are urea based.
3) Yes, the proposed modification is supported. The data presented in the "Evaluation of the Determination of Free Urea in Water-Soluble Liquid Fertilizers containing Urea and Ureaforms by Urease Method and by HPLC Methods" supports the need to provide this clarification.
4) Yes,The authors present significant and sufficient evidence that Official Method 959.03 is not suitable for urea-formaldehyde products. In this situation, HPLC-based methods are preferred, particularly AOAC Official Method 2003-14. It is clear that with one of the major sources of variability in the Official Method 959.03 is the source of urease, which should be further specified.

Does your organization, have any additional suggestions regarding the modification of this method?

1) No
2) No
3) The following 4 typo/transcription type comments are submitted for consideration regarding the "Evaluation of the Determination of Free Urea in Water-Soluble Liquid Fertilizers containing Urea and Ureaforms by Urease Method and by HPLC Methods" document:
   Comment 1: Page 4, line 3 - change "...difference of the unreacted free urea subtracted from total the N, both..." to "...difference of the unreacted free urea subtracted from the total N, both..."
   Comment 2: Page 11, line 4 - update the Table 3 values to reflect changes described in Comment 3.
   Comment 3: Page 12 Table 3. The % Urea-N HPLC Method AOAC 2003.14 (Means from two Labs) entry for Fertilizer Sample 9 should be 11.73 instead of 11.37 (transcribed from Table 2). This would then make the %Urea-N Difference Urease-HPLC (Mean) become -2.84.
   Comment 4: Page 16, line 2 - confirm the data is in Table 5 vs. Table 6.
4) No

EXPERT REVIEW PANEL (ERP) FOR FERTILIZERS

RECOMMENDATION OF PROPOSED MODIFICATION

1) Yes - I agree that the proposed changes can be implemented
2) No – I disagree that the proposed changes can be implemented.
3) Yes - I agree that the proposed changes can be implemented for OMA 932.14.
4) Yes - I agree that the proposed changes can be implemented for OMA 932.14.

Please explain and delineate for scientific reasons

1) The data from the revise method is compared with AOAC Final Action Method 2003.14
2) These modifications do not alter the validated performance of the method.

Please explain the additional revisions:

1) The modifications to the method to validated by comparative data between the revised AOAC OMA 959.03 and 2003.14 which is final action HOLC method

2) On page 1476, in the second paragraph after Experimental-->Materials-->(l), the last sentence "Analytical results, means, and standard deviations are shown in Table 1" should be Table 2.

   On page 1477, the last sentence of Apparatus-->(a) "Instrument operating conditions are listed in Table 2" should be Table 1.

   On page 1480, the last sentence of paragraph 6 "...the Urea-triazone liquid solution containing mixed compositions of urea forms and triazone moieties (Table 1)" should be Table 2.

RECOMMENDATION OF PROPOSED MODIFICATION LEVEL

1) Minor Modification
2) Editorial Change
3) Minor Modification
4) Minor Modification