

The Veterinary Drug Residue Sub-group of the AOAC Community for Chemical Contaminants and Residues would like to propose to the AOAC Official Methods Board that they convene an expert review panel (ERP) to select a method for dye residues in seafood as first action official AOAC method using the new alternative path. Furthermore, the group recommends that the LC-MS/MS method developed by the Anses laboratory in France be given serious consideration as the method to be selected.

I. Background:

A. Stakeholder Panel

A stakeholder panel consisting of representatives from industry, trade groups and government was convened in 2009 for veterinary drugs in seafood. In 2010 the AOAC issued a call for methods for drug residues in shrimp, catfish, tilapia, and salmon for collaboration through the AOAC *Official Methods* program. Residues of interest included nitrofurans, chloramphenicol, quinolones and fluoroquinolones, dyes and methyl testosterone. The following method performance requirements (AOAC SMPR 2009.006) were agreed upon by that group:

5. Method Performance Requirements

Performance Parameters	Types of Drug Residues					
	Chloramphenicol	Nitrofurans [nitrofurazone, nitrofurantoin, furaltidone, fruzolidone (and marker residues, AOZ, AHD, AMOZ and SEM)]	Fluoroquinolones [ciprofloxacin, enrofloxacin, sarafloxacin, difloxacin, danaofloxacin]	Malachite Green, Crystal Violet, leuco crystal violet, leuco malachite green	Methyltestosterone [17-alpha- methyltestosterone]	Quinolones [oxolinic acid, flumequine, nalidixic acid]
Minimum applicable range	0.3 - 1.2 ppb	1.0 - 3.6 ppb	1.0 - 3.6 ppb	1.0 - 3.6 ppb	0.8 - 3.0 ppb	0.5 - 2.0 ppb
Limit of Detection (LOD)	0.15 ppb	0.5 ppb	0.5 ppb	0.5 ppb	0.4 ppb	0.25 ppb
Limit of Quantitation (LOQ)	0.3 ppb	1 ppb	1 ppb	1 ppb	0.8 ppb	0.5 ppb
Precision (SD):	54%	46%	46%	46%	54%	54%
Recovery (R):	40 - 120%	60-115%	60-115%	60-115%	40-120%	40-120%

Notes:

Method Performance Requirements based on fitness-for-purpose criteria in CODEX CRD 19 (Revised Doc. CX/MAS 09/30/07)

* reproducibility target according to CODEX CAC/GL 71-2009 (page 22)

** criteria assume confirmation criteria met 50 % of time

*** criteria based on what is reasonably achievable given current instrumentation capabilities

In response to this call, over 20 methods were submitted to AOAC for consideration. On September 28, 2010, at the annual AOAC meeting, the veterinary drug residues sub-group of the Chemical Contaminants and Residues in Food Community met to discuss these seafood residue methods as well as other selected methods from the literature. At this meeting, it was decided that nitrofurans and dye residue methods would have priority for collaboration. Smaller working groups were formed to evaluate and select specific methods to go forward.

B. Working group

A working group consisting of Steve Lehotay (USDA/ARS), John Reuther (Eurofins), Eric Verdon (Anses/France) and Olga Shimelis (Supelco) met at the 2010 veterinary drug residue subgroup meeting to evaluate the available methods for dye residues. While many methods were determined to be fit-for-purpose and met analytical performance requirements, two methods were considered for the collaborative study based on advantages in speed and ease: 1) USDA/ARS method ¹ and 2) Anses/France method (submitted as an SOP and later published ²). It was decided that the USDA/ARS method would be taken through the collaborative process, perhaps along with the Anses method, as USDA/ARS personnel agreed to take the lead in developing a study protocol. This working group was later expanded to include Guoying Chen (USDA/ARS), Sherri Turnipseed and Wendy Andersen (both of US FDA/Denver) and Anita Mishra (AOAC).

The USDA/ARS method had the advantage of not requiring mass spectrometry as the residues were detected by LC-VIS (malachite green and crystal violet) or LC-fluorescence (leuco malachite green and leuco crystal violet). However, the method did not include brilliant green and had only been developed and validated for one species (catfish), so further laboratory optimization was required to expand the scope of the procedure. Unfortunately, during this process, it was determined that the method would not be suitable for use in salmon as the recoveries for malachite green did not meet the specified method performance criteria.

The other method selected for further consideration was an LC-MS/MS method developed in the Anses laboratory in France. A complete single laboratory validation for this method was completed for trout. Method specificity and matrix effects were also determined for shrimp, salmon, pangasius, and tilapia. These results were published in early 2011. This method utilizes a simple, rapid extraction that would allow for potentially high sample throughput. In addition, proficiency testing has been conducted in the EU for the dye residues in fish, and successful results were obtained from many laboratories using this method (or variations of this method). Eric Verdon presented results from these proficiency studies to the veterinary residues subgroup meeting at the AOAC meeting on September 20, 2011. These results are a good indication that this method can be successfully transferred to other laboratories. For this reason, we feel that this method for dye residues in seafood would be an excellent candidate for ERP consideration as a first official action method under the new AOAC alternative path.

The ERP could also evaluate the other submitted methods for dye residues ³⁻⁷, and procedures that the working group did not initially consider ⁸⁻¹². These methods are included in the attached bibliography.

II. Recommendations for Expert Review Panel

The following is a list of potential experts to serve on the ERP and perhaps participate in a multi-laboratory validation if deemed necessary by the ERP.

Eric Verdon

Position: Head of Vet Drug Residue Department/Anses

Qualifications:

- The Anses laboratory is EU Reference Laboratory for Veterinary Drug Residue Control in Food from Animal Origin.
- Has developed, published ², implemented and transferred an LC-MS/MS method for dye residues in seafood.
- This laboratory has participated and coordinated several proficiency test programs for residues in seafood, including two for dye residues.

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ANSES LERMVD

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Steven Lehotay

Position: Lead Scientist

Qualifications:

- Globally known for his work in the development of the Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method for chemical residues in food.
- As study director, successfully coordinated two official AOAC method trials for QuEChERS (#2002.03 and 2007.01).
- Chemists in his group developed and published methods for dye residues in catfish. ¹

Contact:

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USDA Agricultural Research Service

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Wyndmoor, PA 19038, US

John Reuther

Position: President and Laboratory Director

Qualifications:

- As laboratory director, extensively experienced with international sampling and testing protocols for contaminants in food, including seafood.
- Responsible for growth of Central Analytical Laboratories.

- Served as AOAC Chair for Veterinary Drug Residues Subgroup for Chemical Contaminant and Residues in Food Community.

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Jeffrey Van der Riet

Position: Acting National Manager, Food Safety Science Directorate

Qualifications:

- Manages CFIA laboratory operations.
- Served on AOAC committee on natural toxins and food allergens.
- Has published methods³ for dyes in seafood that are used in CFIA regulatory laboratories.

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Cory Murphy

Position: Chemistry Section Manager - Dartmouth CFIA Laboratory

Qualifications:

- Developed many methods for antibiotics, toxins, and metals in seafood.
- Served as AOAC Chair for Metals Subgroup for Chemical Contaminant and Residues in Food Community.
- Has published methods³ for dyes in seafood that are used in CFIA regulatory laboratories.

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Wendy Andersen

Position: Research Chemist

Qualifications:

- Has published several methods⁴⁻⁷ for dyes in seafood used in FDA regulatory laboratories.

- Served as AOAC Drug and Related Topic Subcommittee Topic Advisor for Malachite Green 2006-2007.
- Coordinated a GLP study of melamine depletion in seafood with US FDA's Center for Veterinary Medicine.

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Mary Carson

Position: Chemist

Qualifications:

- Served as Chair of AOAC Methods Committee for Drugs and Related Topics
- Served as AOAC Chair for Veterinary Drug Residues Subgroup for Chemical Contaminant and Residues in Food Community.
- As Associate Referee, successfully coordinated an official AOAC method (995.09) for tetracyclines in milk.

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Matthew Sharman

Position: Team Lead

Qualifications:

- Head of UK National Reference Laboratory.
- Advisor to the UK Veterinary Residues Committee.
- Member of Top Management Group of Biocop.
- Group has published several methods^{11, 12} for dyes in seafood.

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Other that have expressed an interest in participating in the method validation process:

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Olga Shimelis, Supelco, Olga.Shimelis@sial.com

Michael Young, Waters Corp., michael_s_young@waters.com

Table 1**Dye Methods Submitted to in Response to Call for Methods**
(Available from Anita Mishra of AOAC or from Veterinary Subgroup Chair)

Method#	Title	Matrix	From
3	LC-MS/MS determination of MG and LMG in fish products	eel	APFIC/JSCIQ (China) and Thermo
6 & 8	Determination of triphenylmethane dyes in salmon, shrimp, and aquacultured products	(salmon, shrimp, tilapia)	CFIA
16 & 17	Multiresidue method for the triphenylmethane dyes in fish: MG, CV, and BG	(catfish, trout, basa, salmon, shrimp)	FDA-DEN/ ADRC
21	LMG, LCV, BG	fish	NRL Belgium
24	Determination of 3 triphenylmethane dyes residues and their metabolites in by LC-MS/MS	aquaculture products	LERMVD – AFSSA (Anses)

Bibliography of Selected Dye Residue Methods

1. Chen G, Miao S. HPLC Determination and MS Confirmation of Malachite Green, Gentian Violet, and Their Leuco Metabolite Residues in Channel Catfish Muscle. *J. Agric. Food Chem.* 2010; **58**:7109-7114.
2. Hurtaud-Pessel D, Couëdor P, Verdon E. Liquid chromatography-tandem mass spectrometry method for the determination of dye residues in aquaculture products: Development and validation. *J. Chromatogr. A* 2011; **1218**:1632-1645.
3. van de Riet JM, Murphy CJ, Pearce JN, Potter RA, Burns BG. Determination of malachite green and leucomalachite green in a variety of aquacultured products by liquid chromatography with tandem mass spectrometry detection. *J. AOAC Int.* 2005; **88**:744-749.
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5. Andersen WC, Turnipseed SB, Karbiwnyk CM, Lee RH, Clark SB, Rowe WD, et al. Multiresidue method for the triphenylmethane dyes in fish: Malachite green, crystal (gentian) violet, and brilliant green. *Anal. Chim. Acta* 2009; **637**:279-289.
6. Andersen WC, Turnipseed SB, Karbiwnyk CM, Lee RH, Rowe WD, Madson MR, et al. Quantitative and Confirmatory Analyses of Crystal Violet (Gentian Violet) and Brilliant Green in Fish. *Anal. Chim. Acta* 2008.
7. Turnipseed SB, Andersen WC, Roybal JE. Determination and Confirmation of Malachite Green and Leucomalachite Green in Salmon by using No-charge Atmospheric Pressure Chemical Ionization LC-MSⁿ. *J. AOAC Int.* 2005; **88**:1312-1317.
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9. Halme K, Lindfors E, Peltonen K. A confirmatory analysis of malachite green residues in rainbow trout with liquid chromatography-electrospray tandem mass spectrometry. *J. Chromatogr. B* 2007; **845**:74-79.
10. Scherpenisse P, Bergwerff AA. Determination of residues of malachite green in finfish by liquid chromatography tandem mass spectrometry. *Anal. Chim. Acta* 2005; **529**:173-177.
11. Tarbin JA, Barnes KA, Bygrave J, Farrington WHH. Screening and confirmation of triphenylmethane dyes and their leuco metabolites in trout muscle using HPLC-vis and ESP-LC-MS. *Analyst* 1998; **123**:2567-2571.
12. Tarbin JA, Chan D, Stubbings G, Sharman M. Multiresidue determination of triarylmethane and phenothiazine dyes in fish tissues by LC-MS/MS. *Anal. Chim. Acta* 2008; **625**:188-194.