

## GENERAL REFEREE REPORTS

# Committee on Drugs and Related Topics

## Drugs

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## Summary

Drugs have been, until now, an issue which has had little or no development, in regards to new methods, not because there is no need for them, but mostly because the pharmaceutical industry has had poor participation in AOAC activities. Luckily, this has changed recently. In 2002 at the 116th AOAC Annual Meeting in Los Angeles, a Pharmaceutical Task Force was formed as a result of a pharmaceutical panel discussion meeting. It reflected the total commitment that was achieved from the U.S. Food and Drug Administration (FDA), AOAC, U.S. Pharmacopeia (USP), and industry. AOAC INTERNATIONAL was represented by President Tom Jensen, President-Elect Jim Ault, a number of representatives from the Board of Directors, AOAC Drug Methods Committee representatives, and many AOAC members, while FDA was represented by Center of Drug Evaluation and Research (CDER) and Office of Regulatory Affairs (ORA). USP was officially represented by Valentin Feyns, who in his presentation welcomed the idea behind USP, AOAC, FDA, and industry forming the task force and continuing its goals. Sumit Sen was appointed by AOAC as the co-chair of the task force and Ines Cereijo was named as a member. As Cereijo was asked by this Committee to propose some methods (or groups of methods) which should receive a high priority during the next few years, she felt that this would be a great opportunity for the Pharmaceutical Task Force to come forward and propose methods. During the panel discussion the major area that was highlighted by a number of speakers was the task of identifying USP monographs of pharmaceuticals that were either outdated or needed improvement. Feyns mentioned that the task force should review USP monographs and come up with a list of those monographs that needed to be replaced. During 2002 and 2003, Sen had discussions with Roger Williams, head of USP, and other senior officers from USP (Eric Sheinin and Joe Valentino), regarding AOAC-USP collaborative projects that the task force had proposed. He has discussed in detail these collaborative initiatives with E. James Bradford, Executive Director of AOAC, and Diana Hopkins, Director of Governance and Executive Affairs. They also organized a USP-AOAC meeting held on July 30, 2003, at AOAC in Gaithersburg, MD. This meeting was attended by Williams, Sheinin, Valentino, Ronald Manning, Feyns (USP), E. James Bradford, Diana Hopkins, and Anita Mishra (AOAC). There,

it was proposed that AOAC Pharmaceuticals Task Force could undertake collaborative projects on replacing the existing compendial USP monographs on OTC drugs with stability indicating methods. The AOAC Pharmaceuticals Task Force could also utilize the AOAC's e-CAM program regarding this matter.

AOAC Pharmaceutical Task Force meeting at the Atlanta AOAC Annual Meeting was held Monday, September 15, 2003, from 6:00 p.m.–7:00 p.m. There it was discussed that it would be very interesting to program collaborative studies to improve USP methods, although the cost of these studies is an economic challenge.

### *Atenolone in Combination with Chloralidone*

Discontinued.

### *Dexamethasone in Tablets*

Discontinued.

### *Reserpine and Rescinnamin in Rauwolfia serpentina Powders and Tablets*

Discontinued.

### *Determination of Atropine (l-Hyoscyamine) Sulfate in Commercial Products by Liquid Chromatography (LC) with Detection by UV Absorbance and Fluorescence*

Multilaboratory study. Study Coordinator Ugo R. Cieri, U.S. Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106, E-mail: ucieri@ora.fda.gov.

An LC method with 2 detection systems for determining atropine (*l*-hyoscyamine) sulfate in commercial products (tablets, ophthalmic solution, extended release tablets, and injections) was developed. Atropine (*dl*-hyoscyamine) and *l*-hyoscyamine are the principal constituents of *Atropa belladonna* while it may contain small amounts of scopolamine. The same alkaloids and in approximately the same ratios are found in *Datura stramonium*. Greater amounts of scopolamine are found in *Hyoscyamus niger* and *Scopola carniolica*. Belladonna alkaloids are used as anticholinergic, antimuscarinic, antispasmodic, and mydriatic agents. It is believed that the therapeutic properties of belladonna alkaloids depend primarily on the levo isomers, *l*-hyoscyamine or *l*-scopolamine. That is why *l*-hyoscyamine, being about 100% levo is twice as potent as the racemate atropine, which is composed of equal amounts of the *d* and *l* enantiomers. It has been claimed that atropine is not present in plants but that *l*-hyoscyamine is, which during the extraction process undergoes racemization to give atropine. Several commercial preparations, such as tablets, injections, solutions contain atropine or *l*-hyoscyamine as the sulfate salt soluble in water at the

adult dosage of 0.4 mg. Scopolamine hydrobromide soluble salt is less common in commercial preparations, containing adult dosage of 0.4 mg.

Occasionally a product is labeled to contain all 3 alkaloids, atropine sulfate, *l*-hyoscyamine sulfate, and scopolamine hydrobromide.

Published methods for the analysis of products containing belladonna alkaloids utilize gas liquid chromatography (GLC) or LC (1). Generally the chromatogram obtained shows a peak for scopolamine but a single peak for atropine and *l*-hyoscyamine. The USP has several monographs in which the assay of the alkaloid is done by GLC (2). But for the assay of atropine sulfate injections the USP employs LC with a reversed-phase C18 column and detection by UV absorbance at 254 nm (3). For some products containing also diphenoxylate hydrochloride the USP determines atropine sulfate by LC with a reversed-phase C18 column and detection by UV absorbance at 206 nm (4, 5). Ting (6) published a method for the simultaneous determination of scopolamine, *l*-hyoscyamine, and phenobarbitalin tablets with a reversed-phase C18 column and detection by UV absorbance at 220 nm. In an earlier publication (7) for the determination of atropine (*l*-hyoscyamine) Cieri also employed a reversed-phase C18 column but used fluorescence detection with 255 nm excitation and 285 nm emission. A recent experimental work performed in this laboratory showed that a short normal-phase (silica) column is a valid alternative in the LC determination of belladonna alkaloids. The advantage of this column over some of those previously proposed is that it reaches equilibrium very quickly and remains stable for a long time. Mobile phase chosen has similarities with those of previous LC methods but requires smaller quantities of ion-pair reagents in the aqueous phase. As the other methods reviewed, atropine and *l*-hyoscyamine are not separated forming a single peak. Scopolamine elutes earlier and, if present, is well separated from the combined atropine-*l*-hyoscyamine peak. The samples selected for this study however did not contain scopolamine.

Detection was accomplished at UV 220 nm, as previously done by Ting (6), but also by fluorescence at 255 nm excitation and 285 nm emission, as reported in the previous publication.

This study went under revision for its publication and it was determined that the study does not conform to the requirements of a collaborative study, which include the approval of the study protocol by the appropriate committee (including statistical design and safety reviews), did not include the mandatory analysis of blind duplicates, included only a very limited range of concentrations in the study samples, and did not include required elements of statistical assessment. Consequently, it has been accepted for publication in the *AOAC Journal* as a multilaboratory study.

#### *Interlaboratory Studies*

In Argentina, AOAC together with the Institute for Medicines Department of the Ministry of Health organized the first proficiency testing round for the pharmaceutical industry. The objective of this study was to evaluate the performance of the

28 participant laboratories: 2 governmental, 2 academic, and 24 private (10 multinational and 14 national).

They had to analyze 2 generic drugs, mebendazole and sodium diclofenac, for quantification and loss on drying. The data of the analyses performed by the laboratories were analyzed and identified with a code number. Loss on drying was performed by the gravimetric method and quantification assay was carried out according to the methods described in USP XXV Ed. or Eu. Ph., 4th Ed. (8).

It was decided to utilize robust statistics (3.18. ISO Guide 43-1), in order to include all the results. The trend is to employ, where possible, robust procedures that weigh the results, not affecting them by the presence of anomalous data. The robust techniques minimize the influence of extreme results on the estimation of the median and of the standard deviation. These techniques do not discard extreme values from the set of data, but they do assign to them less weight.

Finally, *z*-score values were determined. The *z*-scores are normalized values that give a punctuation to the determinations carried out by each laboratory relative to those performed by the other laboratories on the same sample. So, the performance of each laboratory was evaluated by means of the *z*-scores between- and within-laboratories.

The criteria of acceptance of the *z*-score (ISO Guide 43-1; 1997) were the following: Satisfactory,  $|z| < 2$ ; Questionable,  $2 < |z| < 3$ ; Nonsatisfactory,  $|z| > 3$ .

A *z*-score near 0 indicates that the determination of the laboratory is in accord with those of the other laboratories while an outlier would be any result which has an absolute value of *z*-score-between laboratories higher than 3, that is to say a nonsatisfactory result.

Of the 27 participant laboratories, only 4 (14.8%) reported one or more nonsatisfactory results. Of the 212 reported analyses, a total of 7 (3.3%) were identified as nonsatisfactory and 13 (6.1%) had a high variability between the 2 replicates of their analyses (*z*-score within-laboratories nonsatisfactory).

The laboratories that used the LC and the CE methods had satisfactory *z*-score values. This fact showed that the method used for the determination of the quantification did not influence the results.

As a consequence, 2 Certified Reference Materials (CRM) were obtained. The consensus values and their corresponding uncertainties were estimated with the medians of all the determinations and their confidence intervals (CI) of 95%.

The results were the following: Consensus value for quantification of sodium diclofenac: 99.78 ± 0.2, CI 95%: (99.65; 99.90); consensus value for loss on drying of sodium diclofenac: 0.25 ± 0.02, CI 95%: (0.23; 0.28); consensus value for quantification of mebendazole: 99.89 ± 0.16, CI 95%: (99.72; 100.05); consensus value for loss on drying of mebendazole: 0.27 ± 0.04, CI 95%: (0.23; 0.30).

Besides the rest of the determinations specified in the pharmacopoeias for each drug were carried out by 2 reference laboratories and were included in the certificate as uncertified values. It would be interesting to point out that these CRM's are the first of their kind carried out worldwide in the pharmaceutical laboratories.

A second round has taken place with another 2 drugs: enalapril maleate and midazolam hydrochloride. This time 33 laboratories participated, and the results are being evaluated at the present moment. As soon as they are handed out, a third round will be organized with the following drugs: amidarone hydrochloride, amlodipine besilate, salbutamol, ciprofloxacin hydrochloride, neomycin sulfate, and polymixin B sulfate. In this last round laboratories from other countries of Latin America will be invited to participate.

#### Collaborative Study

As a result of the second round of the Proficiency Testing, a collaborative study has arisen, in order to improve the quantification method, and is going to be organized. The topic to consider will be *Determination of Enalapril Maleate by HPLC*, Study Director Clyde Carducci, Junin 956, Facultad De Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, E-mail: ccardu@ffy.uba.ar. This drug is currently analyzed according to the *European Pharmacopoeia* (8) by a titrimetric method. Although absolute methods don't need Standard Reference Material, sometimes, according to the nature of the drug it is necessary to have complementary chromatographic methods where all the impurities can be separated.

#### Recommendations

(1) *Determination of Atropine (l-Hyoscyamine) Sulfate in Commercial Products by Liquid Chromatography with Detection by UV Absorbance and Fluorescence*: Study Coordinator Ugo R. Cieri, U.S. Food and Drug Administration, 2nd and Chestnut Sts, Philadelphia, PA 19106, E-mail: ucieri@ora.fda.gov. Discontinue study.

(2) *Determination of Enalapril Maleate by HPLC*: Study Director Clyde Carducci, Universidad de Buenos Aires, Junin 956, Facultad de Farmacia y Bioquímica, Buenos Aires, Argentina, E-mail: ccardu@ffy.uba.ar. Evaluate results of precollaborative study and recommend for collaborative study. Continue study.

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- (3) U.S. Pharmacopeia 26 National Formulary 21 (2003) U.S. Pharmacopeial Convention, Rockville, MD, 193, 942–943, 1669–1670
- (4) U.S. Pharmacopeia 26 National Formulary 21 (2003) U.S. Pharmacopeial Convention, Rockville, MD, p. 192
- (5) U.S. Pharmacopeia 26 National Formulary 21 (2003) U.S. Pharmacopeial Convention, Rockville, MD, p. 641
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## Drug Residues in Foods

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### Summary

The AOAC Research Institute (AOAC-RI) continued to evaluate and validate rapid methods for the detection of antimicrobial residues in foods. This year, it certified the New SNAP Beta-Lactam test kit (#030302) for screening raw, commingled whole bovine milk for 6  $\beta$ -lactam antibiotic drug residues: amoxicillin, ampicillin, ceftiofur, cephapirin, and penicillin G. It also certified another test kit developed by Charm Sciences, the Charm SL-6  $\beta$ -lactam test kit (#040301), for screening commingled bovine milk for residues of amoxicillin, ampicillin, cephapirin, ceftiofur, cloxacillin, and penicillin G. This brings to 9 the total number of commercially available antibiotic test kits that have been certified by the AOAC-RI for screening veterinary drug residues in raw commingled bovine milk since the test kit evaluation and certification program began in the early 1990s.

The Parallax Test kit, certified in 2000 by AOAC-RI for screening 6  $\beta$ -lactam drug residues in bulk tank bovine milk, was adapted for screening the following antibiotic drug residues in bovine and porcine kidneys: tetracycline, oxytetracycline, chlortetracycline, doxycycline, ceftiofur, cefquinome, cephapirin, penicillin G, ampicillin, and amoxicillin (1). All the tetracyclines were detected at 300 ppb, below the Codex recommended maximum residue limit (MRL) for kidney of 1200 ppb; ceftiofur was detected at 1000 ppb in kidney muscle (MRL is 4000 ppb) and cefquinome at 200 ppb; cephapirin was detected at 100 ppb; penicillin G, ampicillin, and amoxicillin were all detected at the recommended Codex MRL of 50 ppb. In its current format, this test kit is unable to detect other commonly used antimicrobials such as the sulfonamides, fluoroquinolones, and macrolides.

The Delvotest SP, also certified by the AOAC-RI in 1993 for bulk tank bovine milk, was successfully used in a study to screen antimicrobials in ewe milk with the aid of a photometric detector (2). In another study, test results obtained with the Delvotest were shown to compare with results obtained with the Disk Diffusion Method when both tests were used to monitor for antimicrobials in milk (3).

The application of surface plasmon resonance (SPR) technique, pioneered by the Swedish company Biacore for veterinary drug residue monitoring and surveillance programs, is becoming increasingly popular. This year alone, at least 6 studies in which the Biacore sensor was used for screening veterinary drug residues in foods have been reported. In one study, the technique was used to detect multiple  $\beta$ -agonist residues (4) in bovine livers and it was demonstrated that malbuterol, clenbuterol, and salbutamol residues in bovine

livers can be detected at concentrations down to 0.02, 0.11, and 0.19 g/kg, respectively, while several other  $\alpha$ -agonists can also be detected at concentrations below 1.5 g/kg. In another study, the technique was used to detect multiple sulfonamides (sulfamethazine, sulfisoxazole, sulfachlorpyridazine, sulfachlorpyrazine, sulfamerazine, sulfadiazine, sulfatroxazole, and sulfathiazole) with detection limits between 7 and 20 g/L in diluted chicken serum (5). The technique has been used to detect nicarbazin, a feed additive used globally to prevent outbreaks of coccidiosis in broiler chickens, poultry liver, and eggs with detection limits of 17 and 19 g/kg, respectively (6). It has also been used to detect residues of the 5 aminoglycosides (gentamicin, neomycin, kanamycin, streptomycin, and dihydrostreptomycin) in skimmed milk powder with detection limits between 15 and 60 g/L; concentrations which are well below the European Union (EU) MRLs defined for these drugs that range from 100 to 500 g/L (7). Another interesting application of the technique was reported for the detection of levamisole in liver and milk (8) with detection limits of 6.8 g/kg and 0.5 g/L, respectively. Levamisole is used widely for the control of gastrointestinal parasites in many animal species. While an MRL of 100 g/kg has been defined by the EU for levamisole residues in liver, there is no MRL defined for milk. Thus, this method provides regulators with a technique for monitoring any extra-label use and/or misuse of this drug in dairy animals. Finally, one study reported that test results obtained using the Biacore sensor for the determination of ractopamine in cattle and sheep urine were slightly higher than with those obtained by LC and enzyme-linked immunosorbent assay (ELISA; 9). It was demonstrated in this study that, unlike the LC method which responded only to the parent ractopamine, the Biacore sensor responds to both parent ractopamine and its glucuronides. The most attractive characteristic of this SPR technique is the simplicity involved in sample preparation and the speed with which sample analysis is accomplished, which is typically 6–8 min per sample.

Analytical methods have, for the past several years, been characterized by parameters such as selectivity, specificity, accuracy, precision, repeatability, analytical range, and detection sensitivities described by the limits of detection (LOD) and quantitation (LOQ). As a result of discussions that have recently taken place within the EU, 2 new parameters, detection capability (CC) and decision limit (CC), have been introduced as analytical parameters that must be defined to provide a better characterization of the LOD and LOQ for a validated analytical method intended for regulatory use within the EU. The decision limit is defined as the limit at and above which it can be concluded with an error probability (1 or 5%) that a sample is noncompliant, while the detection capability is the smallest content of the substance that may be detected, identified, and/or quantified in a sample with an error probability of (5%). As a result of the introduction of these 2 new parameters, methods reported in this review will show calculations for either LODs and LOQs, or CC and CC.

Several new methods were reported for the determination of chloramphenicol (CAP) residues in meat and seafood prod-

ucts. One method described the use of isotope dilution electrospray negative ionization mass spectrometry (MS) combined with liquid chromatography (LC; 10) for the determination of CAP in meat (chicken, turkey, beef, and pork) and seafood (dab, shrimp, and fish in dry powdered form). The method uses liquid-liquid extraction followed by silica solid-phase extraction (SPE) cleanup and mass spectral analysis using multiple reaction monitoring of 2 diagnostic transition reactions for CAP  $m/z$  321 257 and  $m/z$  321 152 together with the transition reactions from the  $^{35}\text{Cl}/^{37}\text{Cl}$  ratio  $m/z$  323 257 and  $m/z$  323 152. The method was validated and a decision limit and detection capability of 0.01 and 0.02 g/kg, respectively, were calculated. Another method was developed for the determination of CAP in bovine, porcine, and chicken muscle tissue (11) by matrix solid-phase dispersion (MSPD) extraction technique followed by high resolution gas chromatographic (GC) analysis of the disilylated trimethyl-CAP and detecting the derivatized CAP with an electron capture detector (ECD). The LOD and LOQ for this method were 1.6 and 4 g/kg, respectively. Another method was reported for the detection and confirmation of CAP in equine, porcine, and bovine muscle and urine (12). This method involved extracting CAP with ethyl acetate, a 2-step C18 SPE cartridge cleanup followed by negative ion atmospheric pressure chemical ionization mass spectrometry (APCI-MS) analysis. The authors demonstrated that with the simple cleanup procedure described, a LOD of 0.02 g/kg was achievable for CAP in urine by LC/MS/MS compared to only 2 g/kg by electron impact GC/MS.

A method for the simultaneous determination of residues of CAP, thiamphenicol, florfenicol, and florfenicol amine by LC/MS was described in which the phenicols were extracted from salmon, rainbow trout, and shrimp with water and acetone (13). The phenicols were back-extracted into dichloromethane (DCM), and the DCM layer evaporated to near dryness. After the residual extract was dissolved in 0.1% acetic acid solution (1 mL), it was washed with hexane, centrifuged at 137 g, and the lower aqueous portion filtered and analyzed by reversed-phase LC/MS. LODs of 0.1, 0.3, and 1.0 ng/g were estimated for florfenicol and CAP, thiamphenicol, and florfenicol amine, respectively. In another method, florfenicol in 2.0 g channel catfish muscle tissue was acid hydrolyzed for 3 h at 100 C followed by ethyl acetate extraction (14). After centrifugation at 1303 g for 5 min, the ethyl acetate layer was aspirated to waste and the pH of the hydrolysate adjusted to >12.5 with 30% (w/w) NaOH. The sample was then cleaned up on a Varian Chem Elut SPE column, dried, dissolved in 2 mL final extract solution, filtered, and analyzed on a reversed-phase Zorbax C8 LC column (250 4.6 mm) with UV detection at 220 nm. LOD of 0.044 g/g and a LOQ of 0.075 g/g were calculated for this method.

A method for the determination and confirmation of CAP in bovine milk by LC/MS/MS was recently reported (15). In this method, 10 mL defatted milk was heated to 380–40 C and cleaned up on a C18 SPE cartridge. The eluate was further cleaned up on a neutral alumina SPE cartridge, dissolved in 25% acetonitrile, filtered, and analyzed by LC-APCI-MS. Confirmation was based on selected reaction monitoring of the

ion transitions  $m/z$  321 152 (100%),  $m/z$  321 194 (35%), and  $m/z$  321 257 (65%). With recoveries of 90% for CAP using this simplified sample preparation procedure, the authors were able to confirm CAP residues in milk at a concentration of 0.1 g/kg. Another method was reported for the determination of CAP together with 6 sulfonamides (sulfamethazine, sulfathiazole, sulfamerazine, sulfachlorpyridazine, sulfamethoxazole, and sulfamonomethoxine) and 3 nitrofurans (nitrofurazone, furazolidone, and furaltadone) in pasteurized milk (16). In this method, 10 mL pasteurized bovine milk fortified with the 10 compounds were extracted with acetone-chloroform and the organic phase was evaporated. The residual extract was dissolved in a 0.02M aqueous sodium acetate buffer (pH 4.8) and washed with hexane. After aspirating the hexane layer to waste, the aqueous layer was filtered and analyzed as follows by reversed-phase LC with UV detection: CAP and the sulfonamides were separated using gradient analysis and detected at 275 nm; the nitrofurans were separated isocratically and detected at 375 nm with minimum detection limits >10 ng/mL for all the sulfonamides and CAP. The EU MRL for total sulfonamides in milk is 100 g/L. Thus, with minimum detection limits >10 g/L for all the sulfonamides and CAP, this method would be suitable for regulatory screening for these sulfonamide drug residues in milk, but not for CAP (a banned substance).

To address regulatory demands by the European Commission for enhanced residue testing for CAP drug residues in certain fishery and aquaculture products from South East Asia into the European Community, an analytical method was developed that was based on ELISA for screening and GC/MS/MS and/or LC/MS/MS for confirmation (17). In this method, ELISA was carried out directly on an aqueous extract of shrimp, or after extraction with ethyl acetate. Confirmation of suspect samples was performed after extraction with ethyl acetate followed by defatting with *n*-hexane. After a C18 SPE cleanup, the extract was derivatized with *N*-methyl-*N*-trimethylsilyltrifluoroacetamide (MSTFA) and analyzed by negative ion GC/MS/MS. Any presumptive positive (suspect) shrimp sample could be confirmed at the 0.1 g/kg level.

Methods continued to be developed for residues of the sulfonamide group of antimicrobial drugs in milk. In one method, a simple, one-step ultrafiltration procedure coupled with LC/MS/MS analysis was developed and validated for the determination of 5 approved sulfonamides (sulfadiazine, sulfamethazine, sulfamethoxazole, sulfadoxine, and sulfadimethoxine), and one (dapson) that is unapproved for use in food-producing animals (18). The validated method, with LOQs of 5 g/L for all the compounds, was shown to be suitable for the determination of both approved and banned sulfonamides. The one-step ultrafiltration procedure, with slight modifications, was demonstrated also to be useful for the quantitation of another class of veterinary drugs, the benzimidazoles and their metabolites, namely, albendazole, albendazole sulfoxide, fenbendazole, oxfenbendazole, and oxfenbendazole sulphane, at concentrations 2 g/L. Another simple, but sensitive method that used a graphitized carbon black sorbent SPE was developed for the determination of 14 sulfona-

mides (sulfamethazine, sulfamethoxazole, sulfaguandine, sulfanilamide, sulfathiazole, sulfapyridine, sulfamerazine, sulfamer, sulfamethizole, sulfamethoxyypyridazine, sulfachlorpyridazine, sulfamonomethoxine, sulfaquinoxaline, and sulfadimethoxine) drug residues in milk and eggs (19). Estimated LOQs of the method were 1–6 g/L in whole milk, and 5–13 g/kg in eggs. A rapid, quantitative and confirmatory method for 12 sulfonamide drug residues in milk and eggs with LOQs of 1–3 g/L for milk and 2–6 g/kg in eggs (20) was reported. In this procedure, 4 mL milk were deposited on sand (cristobalite) and packed into an extraction cell. The sulfonamides were extracted by flowing 4 mL water through the cell heated at 75 C. After the pH of the extract was adjusted, 500 L of the final extract was injected into the LC and analyzed by electrospray positive ion mass spectrometry (ESI/MS). In another study, a polymeric SPE cartridge was used for the cleanup of a milk extract fortified with 6 sulfonamide drug residues at 8.2 g/L prior to analysis by LC-APCI-MS. The method was demonstrated to be suitable for monitoring sulfonamide drug residues at concentrations below the MRL of 10 g/L defined for sulfonamide residues in Korea (21). In this procedure, the pH of 20 mL milk fortified with 6 sulfonamides (sulfamethazine, sulfadimethoxine, sulfathiazole, sulfadiazine, sulfamethizole, and sulfapyridine) was adjusted to 4.5 and extracted with 20 mL acetonitrile. After centrifugation at 1990 g for 5 min, the upper acetonitrile layer was transferred to a 100 mL round bottom flask and the volume of extract reduced to 5 mL by rotary evaporation at 40 C. Then, 100 mL water was added to the sample, loaded onto a conditioned ethylvinyl-divinyl benzene copolymer cartridge, washed with 2 mL water, and eluted with 10 mL acetonitrile. The eluate was evaporated to about 0.5 mL using the TurboVap evaporator, 0.5 mL water was added, and the solution was filtered and analyzed by reversed-phase LC on an ODS-2 column with detection by APCI-MS.

A rapid method with a LOQ of 10 g/kg was used to monitor the persistence or absence of sulfathiazole residues in French honeys by LC with fluorescence detection (22). It was reported that of the 148 honey samples collected in 2001 as part of the French National Residue testing program, 19 samples were found to be contaminated with sulfathiazole residues at concentrations ranging from 10–6127 g/kg.

The tetracycline family of antibiotics continue to be used extensively for food animal and aquacultured fish production. In the European Community (EC), MRLs of 100, 300, and 600 g/kg in muscle, liver, and kidney, respectively, have been defined for total oxytetracycline (OTC) (OTC + 4-epi-OTC) drug residues in order to protect humans from exposure to OTC in edible tissues of animal origin. It has always been hypothesized that the 4-epimer of OTC (4-epi-OTC) was formed in vivo after OTC administration to animals rather than through artifact formation as a result of sample manipulation prior to chemical analysis. To systematically investigate the hypothesis, an LC method that permitted the separation of OTC and 4-epi-OTC on a PLRP-S polymeric reversed-phase column was first developed and validated (23); the separated OTCs were detected on a Finnigan LCQ ion trap mass spec-

trometer equipped with an ESI source operated in the positive ion mode. The procedure was used in a tissue depletion study in which the residual concentrations of OTC and 4-epi-OTC in muscle, kidney, and liver tissues were measured after 16 calves were administered 2 intramuscular injections of a commercial preparation of OTC at a dose of 20 mg/kg body weight. The animals were slaughtered in groups of 4 at 4, 14, 21, and 35 days following drug administration. While residues of OTC and 4-epi-OTC were detected in all the different tissues analyzed, the authors claimed that the concentration of 4-epi-OTC was always lower than that of OTC and below the recommended MRL in all tissues. To answer the question whether 4-epi-OTC detected in the depletion study was formed by *in vivo* epimerization of OTC rather than as a by-product of sample preparation steps, the authors conducted the following 4 experiments: (1) They fortified control tissue samples with only OTC and analyzed the extracts for OTC and 4-epi-OTC. No 4-epi-OTC was detected in any of the tissue extracts. (2) They fortified control tissues with OTC and 4-epi-OTC at the MRL, stored the samples at  $-20^{\circ}\text{C}$ , and analyzed them over a 5 week period. The concentrations of OTC or 4-epi-OTC did not change significantly under frozen storage conditions over this period which was longer than the storage period for the depletion study. (3) They incubated a highly concentrated solution of OTC at  $40^{\circ}\text{C}$  (the approximate body temperature for a calf) for 20 h. While OTC was significantly degraded (about 50%) over this incubation period, no 4-epi-OTC was detected. (4) They stored OTC and 4-epi-OTC standard working solutions at  $4^{\circ}\text{C}$  and found them to be stable for 6 months. In light of these results, the authors concluded that any 4-epi-OTC detected in the tissue depletion study was not formed as a by-product of the sample preparation procedures, but by *in vivo* epimerization of OTC. We can understand why some of the experiments were conducted, but we cannot see how these experiments unequivocally explain the *in situ* formation of 4-epi-OTC.

Survey results from 292 animals (94 cattle and 198 pigs) of residual tetracyclines and sulfonamides in kidneys of diseased animals in the Aichi Prefecture, Japan, over the period 1995–1999 were reported (24). Drug residues found in these animals included chlortetracycline (CTC; 20.2%), OTC (16.1%), sulfamonomethoxine (SMMX; 12.0%), sulfadimethoxine (SDMX; 0.7%), sulfamethoxazole (SMX; 0.7%), sulfamerazine (SMR; 0.3%) and sulfisoxazole (SIX; 0.3%). Concentrations of OTC found in 22 cattle kidneys ranged from 0.05–6.86 and 0.05–3.35 mg/kg in 25 pig kidneys; for CTC this ranged from 0.10–8.93 mg/kg in cattle kidney and 0.10–2.67 mg/kg in pig kidneys. The Japanese MRL of 1.2 mg/kg is defined for total OTC, chlortetracycline (CTC), and tetracycline (TTC), indicating that some of those samples were violative. SMMX residues detected in 7 cattle kidneys ranged from 0.05–0.90 and 0.05–4.84 mg/kg in 28 pigs. SDMX residues found in 2 cattle were 0.69 and 1.70 mg/kg; SMX residues found in 2 pigs were 0.11 and 0.66 mg/kg; SMR and SIX residues were found in one pig at 0.58 and 0.14 mg/kg, respectively. With the exception of sulfadimidine (SDD) for which an MRL of 0.10 mg/kg has

been set, the tolerance for all other sulfonamides is 0 in Japan. All the positive sulfonamide findings are, therefore, considered violative.

Enrofloxacin has been shown to provide high therapeutic efficacy in the treatment of severe systemic infections affecting farm animals and farmed fish, and is currently approved for use in broiler chickens in the United States. Increasing concern for its impact on human health continue to dictate that reliable and sensitive analytical methods be made available for monitoring the persistence of enrofloxacin residues in foods of animal origin. In this regard, a one-step procedure is described (25) in which enrofloxacin is extracted from 2.0 g chicken breast with 6 mL 1% acidic acetonitrile and then analyzed without any further sample cleanup by LC separation on a phenyl column with fluorescence detection (excitation at 324 nm, emission at 442 nm). The method was applied to screen 18 incurred chicken breast samples to determine whether the samples contained enrofloxacin residues at concentrations at, below, or above the FDA tolerance level of 300 g/kg. A method for the determination of 8 fluoroquinolone antibiotics (desethylened ciprofloxacin, norfloxacin, ciprofloxacin, danofloxacin, enrofloxacin, orbifloxacin, sarafloxacin, and difloxacin) in eggs (albumin and yolk) at concentrations ranging from 10–100 g/kg by LC with fluorescence detection and confirmation by ion trap MS (26) was described. Another paper described the use of LC with ion trap MS detection to screen and confirm ciprofloxacin, enrofloxacin, sarafloxacin, and difloxacin residues in salmon muscle tissue (27). Residues of these 4 drugs, at concentrations ranging from 10–40 g/kg, were confirmed in extracts from incurred salmon tissue. A simple multiresidue method for the determination of 13 quinolones (pipemidic acid, rifloxacin, enoxacin, ofloxacin, norfloxacin, ciprofloxacin, danofloxacin, enrofloxacin, difloxacin, cinoxacin, oxolinic acid, nalidixic acid, and flumequine) in feed was developed and validated (28) at 5, 10, and 25 mg/kg feed. In this procedure, feed is extracted with a mixture of orthophosphoric acid–acetonitrile (pH 2.6), cleaned up on an Oasis HLB SPE cartridge, and analyzed by LC on a C<sub>5</sub> analytical column (150  $\times$  4.6 mm id) with fluorescence and/or UV detection.

A method describing the determination and confirmation of halofuginone, a coccidiostat, in chicken liver and eggs was reported (29). An MRL of 10 and 30 g/kg for halofuginone in bovine muscle and liver, respectively, has been established in the EU. There is, however, no MRL established for halofuginone residues in poultry. Since halofuginone is not licensed for use in commercial egg-layers, it is expected that eggs for human consumption must be free from halofuginone residue contamination. This method, with a decision limit of 35.4 g/kg and a detection capability of 43.6 g/kg, was developed and validated for use in a regulatory monitoring program for chicken liver and eggs in the United Kingdom. A relatively fast, simple, and selective LC/MS/MS method for the detection of 5 coccidiostats (diclazuril, dimetridazole, halofuginone, nicarbazin, and robenidine) in eggs (30) was developed and validated. The decision limit for the method ranged from 0.75 to 6.0 g/kg, while the detection capability ranged from 0.9 to 8.3 g/kg.

A method for the determination of 8 anthelmintic drug residues [levamisole (LE), thiabendazole (TB), oxfendazole (OF), oxibendazole (OB), albendazole (AB), fenbendazole (FB), febantel (FE), and triclabendazole (TC)] in milk by LC/MS/MS (31) was reported. MRLs for milk have been established by the European Agency for the Evaluation of Medicinal Products (EMA) for 6 of the 8 anthelmintics: TB (100 g/L), OF (10 g/L), OB (50 g/L), AB (100 g/L), FB (10 g/L), and FE (10 g/L). Since LE and TC are not authorized for use in lactating dairy cows, the authors used the concept of the maximum permitted residue limit (MPRL) of 1 g/L as a guideline for the minimum detection sensitivity of the method that was developed for those 2 analytes. In that method, the pH of a 5 mL sample of milk is adjusted with 100 L 10M NaOH solution, and then extracted with 15 mL ethyl acetate. After centrifugation and evaporation of organic phase to dryness with nitrogen at 50 C, the 8 anthelmintics in the reconstituted extract were separated by gradient analysis on a C18 column and detected by positive ion ESI-MS/MS.

A multiresidue method which used supercritical fluid for the extraction of 10 benzimidazole anthelmintics in ovine, porcine, avian, and bovine liver followed by LC analysis with UV detection (32) was developed and validated with ovine liver fortified at 50, 100, 250, and 500 g/kg. A liver tissue sample is oven dried first and packed into an extraction vessel, rehydrated with water, and extracted at 80 C with 60 L supercritical CO<sub>2</sub> (690 bar). Residues are trapped onto neutral alumina and eluted with 18 mL methanol–water (60 + 40, v/v). The extract is acidified and cleaned up on a strong cation exchange SPE and the dried extract is dissolved in 400 L (1 + 1) methanol–water and analyzed by LC-UV. The method can quantify all the 10 benzimidazole anthelmintics at concentrations 50 g/kg. A method for the determination of mebendazole, the hydrolyzed metabolite, and the reduced metabolite in sheep tissue was developed, validated, and used in a depletion study to monitor mebendazole (MB) and its derived residues from edible tissues of sheep after a single oral administration of the commercial formulation Ovitelmin (33). Samples for analysis were made alkaline and then extracted with ethyl acetate. MB and the metabolites in the tissue extract were separated by gradient elution on a reversed-phase column and detected by positive ESI-MS/MS. Calculated decision limits for MB, its hydroxy metabolite, and the reduced metabolite were 11, 12, and 13 g/kg, respectively; the detection capability for the 3 analytes were 13, 15, and 16 g/kg, respectively. It was found that MB administration left residual concentrations of MB and its 2 metabolites in kidney, liver, and muscle tissues at concentrations that ranged from 21 to 7630 g/kg with the highest residues found in liver. Both the reduced and the hydrolyzed metabolites were still detectable in the liver, and kidney 14 days after drug administration. An LC method with fluorescence detection was developed and validated for the determination of albendazole (ABZ) and its major metabolites, albendazole sulfoxide (ABZ-SO), albendazole sulfone (ABZ-SO<sub>2</sub>), and albendazole-2-aminosulfone (ABZ-2NH<sub>2</sub>SO<sub>2</sub>) in muscle tissues of rainbow trout, Atlantic salmon, and tilapia (34). The

method, with LOQs of 20, 1.5, 0.5, and 5 g/kg for ABZ, ABZ-SO, ABZ-SO<sub>2</sub>, ABZ-2NH<sub>2</sub>SO<sub>2</sub>, respectively, was then successfully applied to the determination of these residues in 3 fish species obtained after oral dosing with albendazole.

A method with a LOQ of 5 g/kg was developed for the simultaneous determination of 6 macrocyclic lactone drug residues (abamectin, doramectin, eprinomectin, ivermectin, milbemectin, and moxidectin) in bovine muscle and liver tissues by LC with fluorescence detection (35). In this method, residues of macrocyclic lactones in muscle and liver tissue samples are extracted with acetonitrile. The extract is partitioned into *n*-hexane, evaporated to dryness, and cleaned up on an amino SPE cartridge. The cleaned up extract is derivatized with *N,N*-dimethylformamide-acetic anhydride-1-methylimidazole, and analyzed by LC with fluorescence detection. In a disposition study (36) to evaluate whether the practice of extra-label use of this macrocyclic lactone to control ecto- and endoparasitic diseases in sheep would result in the persistence of milk residues, 5 female Pampinata dairy sheep with an average weight of 92 kg were given a single dose administration of a commercially available doramectin formulation by subcutaneous injection in the shoulder at 200 g/kg body weight. Blood and milk samples were collected at defined time points and analyzed for doramectin using a validated LC method with fluorescence detection. The disposition data showed that significant concentrations of doramectin residues were still present in plasma (0.48 g/L) and milk (1.03 g/L) 30 days after drug administration.

Flunixin, a nonsteroidal anti-inflammatory drug (NSAID) is approved for use in cattle in the United States. A method was developed for the determination and confirmation of its hydroxy metabolite, 5-hydroxyflunixin, in raw bovine milk using LC/MS/MS (37). With a LOD of 0.2 g/L and a LOQ of 1 g/L, the method was successfully used to confirm the presence of 5-hydroxyflunixin in incurred milk determined to contain 3.2 and 6.8 g/L of the marker residue. Another method was developed for the determination of flunixin, 5-hydroxyflunixin, and ketoprofen in raw milk by LC/MS/MS (38). The method was used in a depletion study in which Holstein-Friesian cows were given either Ketoprofen or Flunixin meglumine (Finadyne) via an intravenous injection at the maximum label dose. Cows were milked twice a day and the milk samples analyzed. Since the highest concentrations found for ketoprofen, flunixin, and 5-hydroxyflunixin were 2.5, 6.7, and 590 g/L, respectively, and the concentration of 5-hydroxyflunixin declined rapidly within a few hours after drug administration to concentrations below the EU MRL of 40 g/mL, it was concluded that the 12 h withdrawal period was appropriate for the intended use.

-agonists continued to attract attention. An interesting case was described in which GC/MS was used to di-

agnose the cause of an alleged food poisoning in 2 patients admitted to a hospital in Portugal with symptoms of food poisoning to be due to eating clenbuterol-contaminated liver (39). Plasma samples taken from the patients within 3 h after ingesting the implicated liver sample were found to contain 60 g/L clenbuterol while the liver sample itself was found to be contaminated with clenbuterol at a concentration of 1.42 mg/kg by GC/MS. A sensitive LC method using fluorescence detection and MS/MS for the routine determination and confirmation of ractopamine residues in animal tissues was developed (40). Bovine and porcine muscle tissue are extracted with methanol and hydrolyzed with  $\beta$ -glucuronidase, followed by liquid extraction and SPE cleanup. Ractopamine was quantified by LC with fluorescence detection using ritodrine as internal standard and confirmed by MS/MS. The method provided recoveries of 80% for ractopamine added to muscle tissue at concentrations of 1–4 g/kg demonstrating that it is useful for screening this drug for which a U.S. tolerance of 0.05 and 0.15 mg/kg, respectively, have been defined for edible swine muscle tissue and liver.

Cattle destined for export to the EU from Canada are required to be certified to have been grown to market weight without the use of hormonal growth promotants (HGP) such as trenbolone (TB), nortestosterone (NT), diethylstilbestrol (DES), dienestrol (DIEN), hexestrol (HEX), and zeranol (ZER), whose use for food animal production has been banned in the EU. To provide this certification, a screening method for residues of TB, epi-trenbolone, NT, and epi-nortestosterone (41) in bovine urine using commercially available immunoaffinity cartridges for sample extraction and cleanup followed by LC-UV analysis was developed and validated at 2.0 g/L, the minimum required performance limit (MRPL) defined by the EU for such methods. An analytical method for regulatory control of banned substances is considered fit for this purpose by the EU if it is able to measure the concentration of the banned substance at MPRL. The same authors combined the immunoaffinity chromatography cleanup procedure with GC/MS to develop and validate a method to screen for DES, DIEN, HEX, and ZER in urine samples at the MPRL (42). A novel procedure for the determination of 17  $\beta$ -estradiol in bovine plasma in which the drug was derivatized with a mixture of pentafluorobenzyl bromide and trimethylsilyltrifluoro-acetamide and analyzed by GC with ion trap MS/MS using methane negative chemical ionization (43) was described. With a method LOD and LOQ of 5 and 20 ng/L, respectively, it was demonstrated that the results obtained with this procedure compared quite well with results obtained on the same samples analyzed by standardized radioimmunoassay techniques. 17  $\beta$ -Estradiol, approved for use in the United States, Canada, Argentina, Australia, and New Zealand, can be used alone or in combination with other natural or synthetic steroid hormones to improve weight gain and feed efficiency in breeding cattle. It is prohibited for use within the EU. Currently, it is nearly impossible to differentiate animals treated with anabolic steroids from nontreated animals. A procedure is described in which high resolution gas

chromatography (HRGC) coupled with high resolution mass spectrometry (HRMS) is used to analyze 17  $\beta$ -estradiol and 17  $\alpha$ -estradiol, their corresponding hydrophilic phase II metabolites (glucurono-, glycosido-, and di-conjugate forms), and lipoidal fatty acid esters in muscle, kidney, liver, and fat samples obtained from control steers and steers administered single or multiple implants of Revalor (44). A multidimensional statistical analysis of the data generated indicated that it is possible to differentiate untreated and implanted steers on the basis of their metabolic profiles using the analysis of 17  $\beta$ -estradiol in fat (free and fatty acid forms), kidney (glucuronide form), and 17  $\alpha$ -estradiol in liver (free form). A method that reports a faster analysis for screening and confirmation of estrogens, gestagens, and androgens in kidney fat by using only 5 g sample size (45) was reported. In this procedure, 5 g kidney fat were extracted with acetonitrile. The extract was washed with hexane, saponified, and cleaned up on an SPE cartridge. The steroids were derivatized and analyzed as their trimethylsilyl ether derivatives by GC with detection by MS/MS.

A validated method was described for the determination and confirmation of 5 glucocorticoid steroids (dexamethasone, betamethasone, flumethasone, fluorometholone, beclomethasone, and triamcinolone acetonide) in bovine urine (46). The method involves an initial enzymatic hydrolysis of the urine sample followed by SPE cleanup and analysis by positive APCI/LC/MS/MS. A decision limit of 2 g/L and a detection capability of 2–3 g/L were calculated for the method. Three calves being treated for respiratory conditions were injected intramuscularly with a commercial formulation of dexamethasone esters (DEX) and slaughtered 72 h after treatment. Another 3 calves being treated for similar respiratory conditions were injected intramuscularly with an aqueous flumethasone (FLU) preparation and slaughtered 24 h post-drug administration. An immunochemical method that had been developed for screening the synthetic glucocorticoids, dexamethasone, and flumethasone in animal tissues (47) was used to analyze residues of DEX and FLU in muscle, kidney, urine, and liver tissues obtained from the diseased calves. The results obtained with the screening test indicated that it was a suitable technique for screening the 2 drugs in tissues and urine samples obtained from slaughter animals. A method was developed for the simultaneous determination and confirmation of betamethasone and dexamethasone residue levels in bovine liver by LC/MS/MS (48). Control liver tissue fortified with the 2 drugs was extracted with acetonitrile and cleaned up on a C18 SPE cartridge. The tissue extract was analyzed on a Hypercarb column and detected by MS/MS. The method was validated and shown to have a LOD of 0.2 g/kg for betamethasone and dexamethasone and a LOQ of 0.4 g/kg for dexamethasone and 0.3 g/kg for betamethasone.

The results of 3 multilaboratory studies were published. In the first study, 4 laboratories located in the United States and Canada evaluated the usefulness of an analytical method developed for the determination of ceftiofur drug residues in bovine milk, kidney, and muscle tissues, and porcine kidney and muscle tissues for regulatory control of the use of ceftiofur in

food animal production practices (49). The study involved incubating 5 mL milk with dithioerythritol (DTE) solution in ammonium acetate buffer pH 8.9 for 15 min at 50 °C to generate desfuroylceftiofur (DFC), an unstable intermediate. The free DFC is extracted and cleaned up on a C18 SPE where it is reacted *in situ* with iodoacetamide to form the stable desfuroylceftiofur acetamide (DCA). After further cleanup on a strong cation exchange SPE column, DCA is separated from tissue and endogenous components by reversed-phase gradient LC with UV detection at 266 nm. For tissue samples, a 1 g tissue sample is fortified at the appropriate concentration with ceftiofur standard solution and incubated with 0.4% DTE in 0.05M pH 9 borate buffer solution for 30 min at 50 °C. The resulting DFC is reacted with iodoacetamide at room temperature for 30 min to form DCA which is cleaned up further as described for the milk samples. Residue results from all 4 participating laboratories obtained on blank, blank-fortified, and incurred milk and/or tissue samples indicated that the method was capable of discriminating uncontaminated milk and tissue samples from contaminated ones as well as differentiating violative (contaminated samples at concentrations > recommended tolerance) samples from nonviolative (contaminated samples at concentrations < recommended tolerance) samples.

In the second multilaboratory study, 14 laboratories located in Europe evaluated the suitability of a multiresidue method developed for the determination of 8 penicillins [benzylpenicillin (PenG), phenoxymethylpenicillin (PenV), ampicillin (AMP), amoxicillin (AMOX), nafcillin (NAF), oxacillin (OXA), cloxacillin (CLOX), and dicloxacillin (DCLOX)] for the analysis of a set of control (drug-free) porcine samples, a set of control porcine samples fortified with the penicillins, and porcine samples containing incurred ampicillin residues at 2 concentration levels of 63.5–9.8 and 358.1–53.4 g/kg (50). Additionally, the 14 participating laboratories were also instructed during the study to use the same methodology to measure the concentrations of PenG, PenV, AMP, and AMOX in control porcine muscle tissues fortified with these 4 penicillins at concentrations ranging from 50 to 300 g/kg. Since only a small number of the participating laboratories reported results on fortified tissues, it was difficult to completely assess the suitability of the method for the other 3 penicillins.

In the third multilaboratory study (51), 18 laboratories located in North and South America, Europe, and Asia evaluated the suitability of an LC method developed for the determination of clopidol residues in chicken muscle tissues by LC with UV detection at 270 nm for regulatory control (52, 53). A total of 306 samples were analyzed that included 36 clopidol-free muscle tissue samples, 54 clopidol-incurred muscle tissue samples, and 216 clopidol-free muscle tissue samples fortified with clopidol at concentrations ranging from 0.10–5.00 mg/kg. The results of the study indicated that the method provides analytical recoveries of 82–85% clopidol added to control chicken muscle tissues at concentrations in the 0.01–5.00 mg/kg range with acceptable reproducibility.

The method was, therefore, recommended and adopted as Official First Action in April 2003.

## Recommendations

(1) *Determination of clopidol residues in chicken tissues*: Study Director Guo-Fang Pang, Qinhuangdao Entry-Exit Inspection and Quarantine Bureau of P.R. China, 39 Haibin Rd, Qinhuangdao 066002, People's Republic of China, Tel/fax: +86 335-340-7608; E-mail: panggfciq@pang.com.cn. Study completed and recommended for Official First Action. Method adopted as Official First Action in April 2003 by AOAC INTERNATIONAL and manuscript has been published (51).

(2) *Determination of streptomycin residues in honey by liquid chromatography*: Study Director Guo-Fang Pang. Any scientist or organization interested in participating in this study is requested to contact the General Referee or AOAC INTERNATIONAL.

(3) *Determination of oxytetracycline, tetracycline, chlortetracycline, and doxycycline residue in honey by liquid chromatography*: Study Director Guo-Fang Pang. Any scientist or organization interested in participating in this study is requested to contact the General Referee or AOAC INTERNATIONAL.

(4) *Aminoglycosides in edible tissues, LC/MS/MS confirmation method*: Topic Advisor Mary Carson, FDA Center for Veterinary Medicine, 8401 Muirkirk Rd, Laurel, MD 20708, Tel: +1-301-827-8169, E-mail: mcarson@cvm.fda.gov. The method developed in the TA's laboratory has been peer-validated by the USDA/FSIS, reviewed, and a manuscript is in preparation.

(5) *Oxytetracycline in shrimp, LC method*: Topic Advisor Phil Kijak, FDA Center for Veterinary Medicine, 8401 Muirkirk Rd, Laurel, MD 20708, Tel: +1-301-827-8166, E-mail: pkijak@cvm.fda.gov. This topic is being transferred from Methods Committee F. A multilaboratory validation study has already been conducted. Kijak is reviewing data and writing the report.

(6) *Chloramphenicol in foods, LC/MS/MS confirmation methods*: Topic Advisor Joanne M. Cook, Florida Dept. of Agriculture & Consumer Services, 3125 Conner Blvd, Lab #3, Tallahassee, FL 32399, E-mail: cookj@doacs.state.fl.us. There has been a lot of activity in this topic area this past year. A number of single laboratory validations have been reported in the literature. A method for seafood has been validated in 3 laboratories, and the manuscript has been accepted for publication by *J. AOAC Int.* A method for honey has also been peer-validated and the manuscript is in preparation.

(7) *Application of optical immunobiosensor technology to screening/determination of veterinary drug residues in animal tissues and biological fluids*: Establishment of new topic and appointment of a Topic Advisor is recommended. Any scientist or organization interested in participating in this study is requested to contact the General Referee or AOAC INTERNATIONAL.

(8) *Evaluation of rapid tests for screening drug residues in animal tissues and biological fluids*: Establishment of new

topic and appointment of a Topic Advisor is recommended. Any scientist or organization interested in participating in this study is requested to contact the General Referee or AOAC INTERNATIONAL.

(9) *Nitrofurans in foods*: Establishment of new topic and appointment of a Topic Advisor is recommended. Any scientist or organization interested in participating in this study is requested to contact the General Referee or AOAC INTERNATIONAL.

(10) *Malachite green in fish*: Establishment of new topic and appointment of a Topic Advisor is recommended. Any scientist or organization interested in participating in this study is requested to contact the General Referee or AOAC INTERNATIONAL.

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