Committee on Drugs and Related Topics

Drug Residues in Animal Tissues

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Summary

The main focus of work surveyed in the past year was in methods for the analysis of antimicrobial compounds.

Two papers were published on the analysis of tilmicosin residues, one dealing with tissues, the other with milk. Following extraction from milk with acetonitrile, tilmicosin is separated from co-extractives using a C18 solid-phase extraction cartridge and analyzed by reversed-phase liquid chromatography (LC) using a 3-component gradient, with detection at 280 nm (1). The method was demonstrated to have a limit of quantitation (LOQ) of 0.010 mg/L for cow's milk and 0.025 mg/L for sheep's milk. Recoveries averaged >80%, with relative standard deviations from 3.1 to 17.2% over the analytical ranges tested (0.010–10 mg/L for cow's milk, 0.025–0.5 mg/L for sheep's milk).

A method for tilmicosin in muscle, liver, kidney, fat, and skin of swine and chickens, and muscle, liver, kidney, and fat of cattle and sheep, was also reported (2). Tissues were initially extracted with methanol and cleanup was performed using a C18 solid-phase extraction cartridge, followed by gradient reversed-phase liquid chromatography, similar to the method for milk. Performance data were reported for the range 0.025–20 mg/kg for all tissues except chicken kidney (0.060–20 mg/kg). Average recoveries ranged from 73 to 98% for the various tissues and concentrations, with relative standard deviations (RSDs) from 0.6 to 14.7%.

The sulfonamides sulfamonomethoxine, sulfadimethoxine, and sulfaquinoxaline were extracted from swine muscle with methanol–water (8 + 2) and then cleaned up on immunoaffinity columns (3). The sulfas were analyzed by reversed-phase LC with UV detection at 270 nm. Recoveries at concentrations from 0.01 to 0.10 mg/kg were 70–94%, with coefficients of variation (CVs) from 3.4 to 12.9%. The limit of detection (LOD) was estimated to be 0.002 mg/kg.

Four different extraction methods were tested for the recovery of residues of chlortetracycline, sulfadiazine, and flumequine from fortified and incurred chicken muscles (4). Although all 4 procedures gave similar recoveries for fortified tissues, higher recoveries were observed from incurred samples when the procedure using a mixer/emulsifier was applied. Analysts should be aware of this in validating methods, and it emphasizes the need to include incurred materials in method validation.

Cephapirin and its metabolites were determined in bovine milk by LC/MS/MS using electrospray ionization with an ion trap mass spectrometric detector (5). The major residues found were cephapirin and desacetylcephapirin, with a LOD of about 1 ng/mL. As the method was intended primarily for qualitative purposes to identify residues present, detailed validation data for quantitative application were not included.

Three extraction procedures were evaluated for the recovery of tetracycline residues from meat meals and from "meat and bone" meals (6). Extraction with HCl following sedimentation of boned particles proved to be the most effective technique. All samples tested (61 meat meals, 26 meat and bone meals) contained detectable residues of tetracyclines.

A survey of residual tetracyclines in kidneys of 147 diseased cattle and 277 diseased swine in Aichi Prefecture, Japan, for the period 1985–1997 was reported (7). The samples, collected from animals that did not pass inspection, were analyzed by LC, using the method that is now Official Method **995.09.** Residues of chlortetracycline (CTC) were found in 64 of the 95 swine kidneys tested which contained tetracyclines; 32 kidneys contained oxytetracycline (OTC). Of the cattle kidneys tested, 36 contained detectable tetracycline residues: 29 with OTC, 5 with CTC, 3 with tetracycline (TC), and 1 with doxycycline (DC). Concentrations for OTC in swine kidney ranged from 0.05 to 33.6 mg/kg; CTC concentrations reported were 0.10–2.76 mg/kg. Residues were lower in cattle kidneys for OTC, ranging from 0.05 to 11.04 mg/kgmg/kg and from 0.10 to 8.93 mg/kg for CTC. Concentrations of TC in cattle kidneys were 0.50–1.67 mg/kg; DC was found at 4.18 mg/kg in the single positive sample.

Interest continued in methodologies for the avermectin and milbemycin classes of compounds that are widely used as endectosides. A depletion study conducted in sheep with moxidectin included validation data for LC detection (8). Following extraction of tissues with acetonitrile, extracts were partitioned and cleaned up on a C18 solid-phase extraction cartridge. A fluorescent derivative was formed by addition of acetic anhydride for LC analysis. Validation data reported for injection site, muscle, liver, and fat included recoveries >75%, RSDs of <10%, and LOQs from 0.08–0.15 ng/g.

An improved method for analysis of eprinomectin in which the addition of acetic acid to the derivatization reagents, resulted in the formation of a stable fluorescent derivative, resolving instability problems previously reported by other authors (9). The procedure modification did not affect method performance for abamectin, doramectin, ivermectin, and moxidectin, which were not affected by the stability problems seen with eprinomectin, usually in the form of low or inconsistent recoveries. The method was validated for residues in bovine liver at 0.004 and 0.020 mg/kg, with recoveries of

73–97%, and RSDs of <15%. This method adaptation has been tested and implemented in the GR's laboratory.

Methodologies for hormonal substances also continue to be of interest. Methodology for the analysis of hormonal substances in implants and tissues from implant sites using LC with diode array detection was applied to the determination of trenbolone acetate, estradiol benzoate, testosterone propionate, trenbolone, zeranol, estradiol, progesterone, and testosterone (10). Tissue samples from implanted animals were homogenized in buffer, extracted with *tert*-butyl-methyl ether and, after additional steps involving decantation, evaporation, and re-dissolving, extracts were applied to a C18 solid-phase extraction cartridge. The hormones were eluted with methanol, which was evaporated. They were then dissolved in acetonitrile for LC analysis on a C18 analytical column (250 × 4 mm), using sequential solvent mixtures as mobile phase. Results were calculated using internal standards. The method was validated at approximately 100 ng/g for trenbolone, zeranol, testosterone, and estradiol and at concentrations from 500 to 1000 ng/g for the remaining compounds. The CVs were <10% for all compounds and concentrations tested during validation.

Following reaction of clenbuterol residues (extracted from swine tissues) with phosgene, it was reported that the inactive dextrorotary isomer was the predominant form present as residues in tissues from swine exposed to this drug (11). Analysis was by GC/MS at concentrations in the low ng/g range. The form of the residues can be of significance not only for toxicological reasons, but also for analysts using immunoaffinity cleanup or enzyme-linked immunosorbent assay (ELISA) if reactivities are isomer-dependent.

The nonsteroidal anti-inflammatory (NSAID) drug flunixin was analyzed in bovine muscle tissue by gradient reversed-phase LC with UV detection at 285 nm (12). Following an enzyme digestion of 2 g homogenized tissue, the digestate was extracted with acetonitrile, transferred through several steps into a phosphate buffer (pH 7.0), and applied to a cartridge containing both C8 and a strong anion exchange material. Flunixin was eluted with acidified hexane, taken to dryness, and dissolved in a solution approximating the mobile phase. The method has an LOD of 6 ng/g and an LOQ of 15 ng/g. Recovery at 100 ng/g was 68.4 ± 5.6%, and RSDs determined at concentrations from 10 to 200 ng/g were <10%. The method was applied to known incurred tissues and to samples submitted by inspection staff (injection sites from 399 antibiotic-suspect animals and a random survey of 335 veal calves). One positive sample was found in the injection sites tested.

There was also continued interest in improved methods for some of the older drugs that are still widely used as coccidiostats and anthelmintics. Amprolium and halofuginone were detected in chicken muscle and eggs by LC with UV detection after extraction with acetonitrile and cleanup on alumina solid-phase extraction cartridges (13). Separation was achieved on the reversed-phase LC column using ion-pairing with sodium 1-heptanesulfonate. The LODs for both amprolium and halofuginone in chicken muscle were 0.03 mg/kg, and 0.04 mg/kg in egg. Recoveries for halofuginone at 0.5 mg/kg were 75 ± 18% in chicken muscle and 55 ± 3% in egg; corresponding results for amprolium were 94 ± 5% for muscle and 85 ± 2% for egg.

Flubendazole and its hydrolyzed and reduced metabolites were extracted from eggs and poultry muscle with ethyl acetate following the addition of 0.1N sodium hydroxide (14). Following further cleanup, including defatting, extracts were analyzed by LC/MS/MS, using reversed-phase LC for separation. Validation followed European Union criteria, at concentrations from 0.2 to 0.8 mg/kg, with recoveries >76% from eggs and >89% from muscle for flubendazole and its 2 metabolites. The LOQ was 0.001–0.002 mg/kg for each of the 3 compounds in egg or muscle, with repeatability of about 10%. Method performance was demonstrated on incurred tissues obtained from a depletion trial conducted in turkeys.

Electrospray LC/MS was used in 2 studies of nicarbazin residues. Validation results were reported for application to chicken muscle and liver (recoveries about 100% at 0.010 mg/kg), but full method details were not included in the paper, which was an application of the method in a residue investigation (15). The method was also applied in a study of nicarbazin residues in eggs from treated chickens (16).

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